MECHANISM OF INTERFERON THERAPY OF MULTIPLE SCLEROSIS: STUDIES IN AN ANIMAL MODEL

By

MUSTAFA GHULAM MUJTABA

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1999

ACKNOWLEDGMENTS

I would like to thank my mentor, Dr. Howard M. Johnson, for taking me into his laboratory and guiding me through my studies. He has always been knowledgeable and logical, which make him a great mentor. I must also express my thanks to my committee members -- Dr. Edward Hoffman, Dr. Jake Streit, Dr. Janet Yamamoto, and Dr. Julie Maupin -- for their time, patience, and effort. Many thanks to my fellow graduate students and labmates both past and present, including Barbara, Jeanne, Brian, Prem, Taishi, Martez, Pedro, Amy, George, Joe, Kendra, Scott, Karrie, Wiggins, and Tim. They make the lab more "interesting" and fun, at least at certain times.

Finally, I thank my family for their support through my graduate school studies. My parents, Ghulam and Uzra Mujtaba, have always been there for me. I thank my brother and sisters and their families for their help and encouragement. Many thanks go to my Afghan cousins whom are always a great break from my graduate studies. I owe a great debt of gratitude to my wonderful family and friends.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

MECHANISM OF INTERFERON THERAPY OF MULTIPLE SCLEROSIS: STUDIES IN AN ANIMAL MODEL

By

Mustafa Ghulam Mujtaba

August 1999

Chairperson: Howard M. Johnson
Major Department: Microbiology and Cell Science

Interferon (IFN) tau is a type I IFN that was originally identified as a pregnancy recognition hormone produced by trophoblast cells. It is as potent an antiviral agent as IFNα and IFNβ, but lacks the toxicity associated with high concentrations of these IFNs in tissue culture and in animal studies. Previously it has been shown that interferon τ pretreatment inhibits the development of both acute and chronic mouse experimental allergic encephalomyelitis (EAE), an animal model for the human demyelinating disease multiple sclerosis (MS). Here, we show that IFNτ induced remission in SJL/J mice that had ongoing chronic active EAE disease, and protected mice against secondary relapses. IFNτ treatment reversed lymphocyte infiltration and microglial activation in the central nervous system. Mice that were treated with IFNτ had lower levels of anti-MBP (myelin basic protein) antibodies than untreated mice in both chronic and acute forms of EAE.

MBP induced proliferation in B cells from EAE mice, but treatment with IFNτ either *in vivo* or *in vitro* blocked this activation. Furthermore, IFNτ inhibited MBP activation of T cells from EAE mice. Thus, IFNτ inhibits the humoral arm as well as the cellular arm of the autoimmune disease EAE. IFNτ prevents EAE in mice by induction of suppressor cells and suppressor factors. Suppressor cells can be induced by IFNτ in tissue culture and *in vivo* by either intraperitoneal injection or by oral administration to mice. Incubation of suppressor cells with MBP-sensitized T cells blocked or delayed the MBP-induced proliferation. Further, intraperitoneal injection of suppressor cells into mice blocked induction of EAE by MBP. Suppressor cells possessed the CD4 T cell phenotype, and produced soluble suppressor factors that inhibited MBP activation of T cells from EAE mice. The suppressor factors were found to be IL-10 and TGFβ, which acted synergistically to inhibit the MBP activation of T cells from EAE mice. These findings are important for understanding the mechanism(s) by which type I IFNs protect against autoimmune disease.

CHAPTER 1
INTRODUCTION

Overview

Interferons (IFNs) are a group of glycoproteins that play important modulatory roles in the vertebrate immune system. Originally discovered in 1957 for their ability to interfere with viral replication in cells (Isaac and Lindenmann, 1957), they are now known to also have antiproliferative effects on a variety of cell types. For this reason, IFNs have been used for the treatment of autoimmune diseases, viral infections, and several types of cancers (Gutterman *et al.*, 1994).Interferon-tau (IFNτ) is a type I IFN that was originally identified as a pregnancy recognition hormone produced by trophoblast cells in sheep. It is as potent an antiviral agent as IFNα and IFNβ, but lacks the toxicity associated with high concentrations of these IFNs in tissue culture and in vivo (Bazer *et al.*, 1989; Pontzer *et al.*, 1991; Soos *et al.*, 1995a; Soos *et al.*, 1995b).

Multiple Sclerosis (MS) is one of the most common disease of the central nervous system (CNS). In MS, the loss of myelin is accompanied by a disruption in the ability of the nerves to conduct electrical impulses to and from the brain, and this produces the various debilitating symptoms of MS. Experimental allergic encephalomyelitis (EAE) is a murine model useful for studying the demyelinating disease MS. Myelin basic protein (MBP) has been shown to be one of the primary CNS antigens responsible for induction of autoimmunity in the EAE model (Zamvil *et al.*, 1990). Immunization of mice with MBP results in tail and limb paralysis due to lymphocytic infiltration and demyelination in the CNS (Zamvil *et al.*, 1990). MBP-specific antibodies and autoreactive MBP-specific T cells are also thought to contribute to the exacerbation of EAE and MS (Sun, 1993; Warren *et*

al., 1993; Gerritse *et al.*, 1994; Saoudi *et al.*, 1995; Wang *et al.*, 1995). Various inflammatory cytokines like tumor necrosis factor-alpha (TNFα) and IFNγ contribute to the break down of myelin on nerve cells in the CNS. Furthermore, microglia, the resident macrophage of the CNS, are thought to play a key role in recruiting lymphocytes and function as antigen-presenting cell in MS and EAE (Benveniste, 1997; Streit *et al.*, 1995; Hickey *et al.*, 1988).

Currently, the type I IFN, IFNβ, is the only FDA approved cytokine treatment of MS. Previously, it has been shown that administration of IFNτ to mice at the time of immunization with MBP blocked the development of EAE in mice without associated toxicity; however the mechanism of such action has not been fully elucidated (Soos *et al.*, 1995a). Thus, the purpose of this study is to determine the mechanism by which IFNτ suppresses autoimmune responses in EAE.

Multiple Sclerosis

Multiple Sclerosis (MS) is a chronic, demyelinating, inflammatory disease of the CNS. MS commonly affects young adults and mostly women (Ebers et al. 1986). MS is commonly found in Canada, the United States, South America, and Europe; near the equator MS is unknown (Ebers *et al.*, 1986). In this disease there is an inflammation of myelin, which is fatty insulation or covering of nerve cell extensions, known as axons (Lassmann, 1998). Messages are sent along axons to other nerve cells in a kind of electrical signals. Myelin insulates the axons to help get these electrical impulses through. It stops currents from flowing between the individual axons. It also helps to speed the conduction of the electrical signal. This enables people to move almost without thinking. When myelin is affected in MS, impulses travel over the axons very slowly, if at all, and there is some electrical interference between axons. Messages are not sent efficiently and can fail to get through so that a person loses the ability to make smooth, rapid, and

coordinated movements (McFarland, 1998). Inflammation in the CNS destroys myelin and the oligodendrocytes, the myelin-producing cells (Mews *et al.*, 1998). After tissue destruction a scar or hardening area forms. These areas are multiple within the CNS, thus the term multiple sclerosis.

The clinical course is variable, but the most common form is characterized by relapsing neurological deficits. Early MS lesions are characterized by local accumulation of activated T cells around small venules (Hauser *et al.*, 1983). Later myelin degeneration occurs associated with perivascular inflammation consisting of T cells, B cells, plasma cells, and macrophages (Prineas *et al.*, 1975; Prineas *et al.*, 1978). T cells are also found at the leading edge of plaques, and they extend into the surrounding normal appearing white matter. The T cells express activation molecules on their cell surface, such as IL-2 receptors and class II major histocompatibility complex (MHC) antigens (Hoffman *et al.*, 1986). In addition, class II MHC expression can also be detected on infiltrating macrophages and resident CNS cells, including microglia, astrocytes, and brain capillary endothelial cells (Hoffman *et al.*, 1986). Class II MHC expression in the CNS is presumably induced by IFNγ secreted by activated T cells. These findings indicate that in early acute MS lesion, demyelination occurs in the face of an active immune response within the CNS. Gliosis is also a prominent feature of MS; this process is characterized by astrocyte proliferation and hypertrophy (Bignami *et al.*, 1972). This reaction eventually leads to the formation of dense glial scars in the CNS, which can contribute to motor and sensory impairment. TNFα, a proinflammatory cytokine, appears to contribute to this process (Salmaj *et al.*, 1990).

The primary demyelination observed in MS results from damage to the myelin sheath or to the myelin-producing cells, the oligodendrocytes. Because myelin is critical for saltatory excitation along axons, demyelination leads to loss of neurological function. Recent findings with magnetic resonance imaging (MRI) indicate that considerable subclinical disease occurs, and that there is breakdown of the blood-brain barrier (BBB)

early in lesion development (Thomson *et al.*, 1992; Gay *et al.*, 1991), which may be a crucial event in the pathogenisis of new lesions in MS. A large body of experimental evidence implicates immune mediated processes in activation and progression of MS. In addition, both genetic and environmental factors contribute to disease (Ebers *et al.*, 1986; Martin *et al.*, 1992). Although the pathological lesions, or plaques, are confined to the white matter of the brain and spinal cord, studies of cerebrospinal fluid and peripheral blood lymphocytes provide evidence for both local and systemic activation of the immune system (Hafler and Weiner, 1989).

IFNγ and TNFα and β have been implicated in exacerbating MS. The ability of TNFα to mediate myelin and oligodendrocyte damage *in vitro* (Selmaj *et al.*, 1988), and its ability to cause cell death of oligodendrocytes *in vitro* (Robbins *et al.*, 1987) may contribute directly to myelin damage or the demyelination process observed in MS. Both TNFα and TNFβ can cause death of oligodendrocytes, the myelin-producing cells of the CNS (Robbins *et al.*, 1987; Paul and Ruddle, 1988).

MS is characterized by migration of inflammatory cells from blood into the brain and subsequent invasion of the extravascular tissue (Cross *et al.*, 1990; Raine *et al.*, 1990). Recent studies have shown that up-regulation of adhesion molecules, such as intercellular adhesion molecules (ICAM-1) on brain endothelial cells by exposure to proinflammatory cytokines such as TNFα and IFNγ mediate leukocyte adhesion to endothelium (McCarron *et al.*, 1993; Fabry *et al.*, 1992; Wong *et al.*, 1992). The presence of ICAM-1 and other adhesion molecules in the vessel walls as well as on astrocytes may guide inflammatory leukocytes into and through the brain, thereby contributing to impairment of the blood brain barrier and the neuropathology of MS.

IFNγ is considered the most potent inducer of class II MHC antigen expression on most cell types, including astrocytes and microglia (Cogswell *et al.*, 1991; Wong *et al.*, 1984; Fierz *et al.*, 1985; Fontana *et al.*, 1984; Pulver *et al.*, 1987; Suzumura *et al.*, 1987). There is little or no expression of class II molecules in normal brain; however IFNγ

induces them on astrocytes and endothelial cells, which can then present myelin antigens to T cells (Fierz *et al.*, 1985; McCarron *et al.*, 1986). Other function of IFNγ that may be important in MS include activation of macrophages, which act as effector cells in demyelination (Bever and Whitaker, 1985) and induction of adhesion molecules, which mediated homing of lymphocytes to sites of inflammation and may facilitate their entry into the CNS (Male *et al.*, 1990). Furthermore, IFNγ-positive cells have been detected in the CNS of patients with MS (Hofman *et al.*, 1991; Traugott and Lebon, 1988). Recent studies on the association of IFNγ with MS demonstrated that peripheral blood lymphocytes from patients with MS produce significantly more IFNγ than those of normal lymphocytes (Beck *et al.*, 1988; Hirsh *et al.*, 1985).

Accumulating evidence supports the notion that MS is an autoimmune disorder mediated by T cells. This evidence includes the pathology of MS lesion, immunological abnormalities in both the periphery and CNS of patients with MS, immunoglobulin synthesis within the CNS, exacerbation of disease after treatment with IFNγ, putative autoantigens such as myelin basic protein (MBP), and involvement of cytokine networks (Martin *et al.*, 1992). Studies have also shown the presence of autoantibodies specifically bound to disintegrating myelin around axons in lesions of acute MS (Genain *et al.*, 1999). Furthermore, peripheral blood T lymphocytes from patients with MS were activated by various MBP peptides (Baxenvanis *et al.*, 1989). Thus, many factors are involved in the pathogenesis of MS.

Experimental Allergic Encephalomyelitis

The best-characterized experimental model for MS is experimental allergic encephalomyelitis (EAE). Similarities shared between EAE and MS are relapsing and chronic paralysis, CNS demyelination, linkage to MHC class II, CD4 T cells present in perivascular inflammatory lesions, and similar autoantigens. MS is a spontaneous disease

in humans, but EAE is induced by injection of spinal cord components such as myelin basic protein (MBP) with adjuvant or transfer of encephalitogenic MBP-specific T cells to naive recipients (Swanborg, 1995; Paterson and Swanborg, 1988; Tabira, 1988, Pettinelli and McFarlin, 1981). MBP is a predominant protein present in myelin in the CNS. Transgenic mice have been constructed that mimics MS in its spontaneous induction and pathology (Governam *et al.*, 1993). EAE can be induced in a number of species including mice, rats, guineas pigs, monkeys, sheep, dogs, and chickens (Sturart and Krikorian, 1928). Clinical signs of EAE include dramatic weigh loss, weakness of the tail and hind limbs, and ascending paralysis. The earliest clinical signs of CNS dysfunction are closely associated with formation of perivasuclar cellular infiltrates and edema with the CNS (Raine *et al.*, 1984; Leibowitz and Kennedy, 1972; Claudio *et al.*, 1990; Cross *et al.*, 1990; Raine *et al.*, 1990; D'Amelio *et al.*, 1990). Demyelination appears to be a later event (Raine *et al.*, 1990; D'Amelio *et al.*, 1990) and may account for chronic neurological dysfunction. EAE is characterized by inflammatory infiltration of the CNS by activated T cells and macrophages, de-myelination, and acute, chronic, or chronic-relapsing paralysis. The mediators of this disease are CNS antigen reactive CD4 T cells that are class II MHC-restricted (Zamvil and Steinman, 1990). Lymphocytes, as mediators of EAE, were first implicated by experiments in which anti-lymphocyte antibodies inhibited induction of EAE (Waksman *et al.*, 1961). Further evidence that T cells were involved stemmed from the observation that thymocytes are required for EAE induction (Arnason *et al.*, 1962). Furthermore CD4 T cells are present in inflammatory EAE lesions in the CNS (Traugott *et al.*, 1986). Most encephalitogenic T cells are of the Th1 subtype, which secrete IFNγ, IL-2, TNFα, and TNFβ (Mosmann and Coffman, 1989). It has been suggested that TNFα and TNFβ secretion by MBP-specific T cell clones correlates with their encephalitogenic potential (Powell *et al.*, 1990). Expression of adhesion molecules also influences the pathogenicity of encephalitogenic T cells (Baron *et al.*, 1993; Kuchroo *et al.*, 1993). Susceptibility to EAE appears to be linked to MHC alleles, although non MHC genes may

have a small role in contributing to EAE (Gasser *et al.*, 1973). Although EAE in animals is initiated by T cells that recognize myelin antigens in the context of class II MHC molecules (Wekerle *et al.*, 1986), some studies have suggested that B-cell activation and antibody responses are necessary for the full development of EAE (Brosnan and Raine, 1996; Willenborg and Prowse, 1983; Piddlesden *et al.*, 1993), and earlier studies on immune-mediated demyelination using myelinated cultures of CNS tissue have indicated that humoral factors are effector mechanisms (Raine and Bornstein, 1970; Raine *et al.*, 1973). Autoantibodies against CNS antigen myelin/oligodendrocyte glycoproteins were identified to bind disintegrating myelin around axons in lesion of acute MS and the marmoset model of EAE (Genain *et al.*, 1999).

Cytokines have been implicated in contributing to EAE disease progression, as well as mediating recovery from disease. Inflammatory cytokines released within the CNS may contribute to the disease process by influencing vascular permeability, inflammatory cell extravasation, and antigen presentation (Mantovani and Dejana, 1989; Martiney *et al.*, 1990; Fierz *et al.*, 1985). Cytokines released within the CNS in response to acute inflammation may also contribute to chronic damage associated with reactive gliosis of astrocytes (Giulian and Lachman, 1985; Selmaj *et al.*, 1990; Yong *et al.*, 1991) and the destruction of oligodendrocytes and myelin (Selmaj and Raine, 1988; Selmaj *et al.*, 1991). The cytokines, IL-1, TNFα, and TNFβ, contribute to the initiation and/or disease progression of EAE (Mannie et. al., 1987; Symons *et al.*, 1987; Jacobs *et al.*, 1991; Ruddle *et al.*, 1990; Selmaj *et al.*, 1991; Kuroda *et al.*, 1991). Studies involving the role of IFNγ show conflicting results. Some studies show that IFNγ plays a protective role in different models of EAE (Billiau *et al.*, 1988; Duong *et al.*, 1992; Voorthuis *et al.*, 1990). Other studies show the detection of IFNγ before the onset of clinical disease (Kennedy *et al.*, 1992; Merril *et al.*, 1992; Stoll *et al.*, 1993) and during the acute phase of disease (Khoury *et al.*, 1992; Baker *et al.*, 1991; Kennedy *et al.*, 1992). Other cytokines such as IL-10, TGFβ, and IL-4 have a protective effect on EAE. TGFβ can inhibit IFNγ-induced

class II MHC antigen expression on both human astroglioma and rat astrocyte cells (Zuber *et al.*, 1988; Schluesener, 1990) and can act as a chemotactic agent for both rat astrocytes and microglia (Morganti-Kossmann *et al.*, 1992; Yao *et al.*, 1990). Microglia, the resident macrophages of the brain, are thought to play a key role in recruiting lymphocytes and function as antigen-presenting cells in MS and EAE (Benveniste *et al.*, 1997; Streit *et al.*, 1995; Hickey *et al.*, 1988). TGFβ inhibits the production of TNFα by microglia (Suzumura *et al.*, 1993) and astrocytes (Benveniste *et al.*, 1994), and TGFβ has also been shown to be an important mediator of oligodendrocyte differentiation (McKinnon *et al.*, 1993). Also, IL-10 has been detected in the CNS of SJL/J mice during disease recovery (Kennedy *et al.*, 1992). IL-4, IL-10, and TGFβ share some similar biological activities because they are all capable of inhibiting secretion of proinflammatory cytokines (Bogdan *et al.*, 1992; Chao *et al.*, 1993).

The EAE model has been used in several novel immunotherapy experiments including anti-TCR antibodies, anti-MHC antibody, anti-CD4 antibody, peptide and interferon therapies, and T cell vaccination (Steinman *et al.*, 1983; Brostoff and Mason, 1984; Howell *et al.*, 1989; Bandenbark *et al.*, 1989; Soos *et al.*, 1997). Thus, the development and testing of a safe therapy for EAE and understanding the mechanism(s) of the therapeutic are the first steps toward identifying potential therapies for MS.

Interferons

Interferons (IFN) are glycoproteins that are produced and released from virally infected cells; they were originally characterized for their antiviral properties (Pestka *et al.*, 1987), and they were first described in 1957 by Issacs and Lindemann (Issacs and Lindeman, 1957). IFNs have been found in all higher vertebrates including humans. They have molecular weights ranging from 15 to 30 KDa (Gastle and Huber, 1988). The idea that IFNs alter the pathogenesis of natural virus infections was supported by observations

that virus-infected animals injected with antibody prepared against IFNs succumbe more rapidly to disease than when virus was given alone (Gresser *et al.*, 1976). IFNs inhibit the growth of viruses of all type in vivo and in vitro. Some viruses are more sensitive to IFN action than others (Grossberg, 1972). Besides their antiviral activities, IFNs possess many other activities including those which are antimicrobial, antitumor, and immunomodulatory (Pestka *et al.*, 1987). IFNs are currently approved by the Food and Drug Administration for a number of diseases such as hairy cell leukemia, condyloma acuminatum, acquired immune deficiency syndrome (AIDS) related Kaposis's sarcoma, chronic hepatitis B and C, gebutak warts caused by papillomavirus, chronic granulomatous disease, and MS (Dorr, 1993; Johnson *et al.*, 1994).

Different IFNs are distinguishable based upon their cellular source, immunological reactivity, and induction of biological responses. There are two main types of IFN, type I and type II. The type I IFNs include IFNα, IFNβ, IFNω, and IFNτ. The type II IFN refers to IFNγ. IFNα and IFNω are produced by leukocytes, while IFNβ is produced by fibroblasts, and IFNτ is primarily produced by the trophoblast cells of the conceptus. IFNγ is produced by T cells and natural killer cells (Baron *et al.*, 1991). IFNγ appears to have a dominant immunoregulatory role while IFNα and IFNβ tend to mediate more antiviral activity. Both type I and type II IFNs have potent antiproliferative effects, while only IFNγ, and not the type I IFNs, can upregulate MHC class II (Houghton *et al.*, 1984, Schwartz *et al.*, 1985). Twenty-six IFNα genes with common structures have been identified, and they encode for at least 22 distinct proteins consisting of 20KDa single polypeptide chains (Zoon *et al.*, 1992). These different IFNαs mediate distinct biological activities in different cells. In contrast, there is only a single form of IFNβ encoded by a distinct gene located next to the IFNα locus in both human and mice (Farrar *et al.*, 1993). Also, a single gene has been identified and described for IFNγ, whereas, several genes for IFNω and IFNτ have been identified (Sen and lengyel, 1992; Bazer and Johnson, 1991).

The type I IFNs bind to a common receptor, whereas IFNγ binds to a different receptor (Langer and Pestka, 1988). IFNγ is considerably more active as an immunomodulator than other classes of IFNs, but its antiviral activity is lower than these other classes. IFNγ is produced by all CD8+ T cells populations and by the Th1 and Th0 subsets of CD4+ T cells following antigenic or T cell mitogen stimulation (Farrar *et al.* 1993). IFNγ can upregulate the surface expression of MHC class I and class II antigens on a variety of cell types both in murine and human (Baron *et al.*, 1991; Sen and Lengyel ,1992). IFNγ production from T cells or NK cells is stimulated by IL-12, which can act synergistically with alloantigens, mitogens, or IL-2 (Stern *et al.*, 1990). In contrast, IL-10 inhibits IFNγ production by T cells and NK cells (Fiorentino *et al.*, 1989).

IFNτ has only been recently described. IFNτ was discovered originally as a pregnancy recognition hormone that is essential for establishment of pregnancy in ruminants such as sheep and cows (Bazer and Johnson, 1991). It was identified as a member of the type I IFN family when it was shown to share significant amino acid sequence homology with the type I IFNs, IFNα and IFNω (Imakawa *et al.*, 1987). IFNτ possesse antiviral, antiproliferative, and immunomodulatory activities similar to the other type I IFNs (Pontzer *et al.*, 1988; Bazer *et al.*, 1989; Soos and Johnson, 1995b). IFNτ differs from the type I IFNs in that it has been shown to be remarkably nontoxic when used at high concentrations in culture and animal studies (Bazer *et al.*, 1989; Soos *et al.*, 1995a; Pontzer *et al.*, 1991), not readily induced by virus and dsRNA (Roberts *et al.*, 1992), and is under genetic regulation different from other type I IFNs (Cross and Roberts, 1991). However, IFNβ, which has been shown to ameliorate relapsing-remitting nature of MS (IFNβ Multiple Sclerosis Study Group, 1993), is currently the only cytokine approved by the FDA for treatment in MS. The mechanism of this amelioration is not fully understood. Therefore, the data recounted here on the mechanism of therapy of EAE by IFNτ may serve as the basis for understanding how type I IFNs may exert therapeutic effects in autoimmune diseases such as MS.

CHAPTER 2
IFNτ INDUCES STABLE REMISSION IN CHRONIC EAE

Introduction

Interferon-tau (IFNτ) is a member of the type I IFN family but unlike IFNα and IFNβ, IFNτ lacks toxicity at high concentrations *in vitro* and when used *in vivo* in animal studies (Bazer *et al.*, 1989; Pontzer *et al.*, 1991; Soos and Johnson, 1995b, Soos *et al.*, 1995b). Like the type I IFNs, IFNτ is acid stable and exerts antiviral, antiproliferative and immunomodulatory activities (Bazer *et al.*, 1989; Pontzer *et al.*, 1991; Soos and Johnson, 1995b). Previously, we have shown that IFNτ blocks the development of acute and chronic experimental allergic encephalomyelitis (EAE) in mice without any associated toxicity (Soos and Johnson, 1995b). EAE is a murine model used to study the human demyelinating disease multiple sclerosis (MS) (Zamvil and Steinman, 1990). In the EAE model, immunization of mice with central nervous system (CNS) antigens such as myelin basic protein (MBP) and proteolipid protein (PLP) results in tail and limb paralysis due to lymphocytic infiltration and demyelination in the CNS (Zamvil and Steinman, 1990).

Treatment of mice with IFNτ before, during, and shortly after immunization with MBP prevents the onset of EAE (Soos *et al.*, 1997). This protection is mediated by IFNτ through induction of CD4 T cells to produce IL-10 and TGFβ which act synergistically to inhibit activation of MBP-sensitized T cells from NZW mice (Mujtaba *et al.*, 1997). Here we demonstrate that treatment of SJL/J mice with IFNτ after the induction of chronic EAE induces them into stable remission. Furthermore, the histological features of the CNS normally associated with EAE such as lymphocyte infiltration and activation of microglia could not be found in the IFNτ treated mice. Microglia, the resident macrophages of the brain, are thought to play a key role in recruiting lymphocytes and function as antigen

presenting cells in MS and EAE (Benveniste, 1997; Streit and Kincaid-Colton, 1995; Hickey and Kimura, 1988). Microglia are process-bearing when they are in the inactivated resting state, but they undergo cell hypertrophy upon activation, and they transform into rounded brain macrophages after neuronal degeneration (Streit *et al.*, 1988). Furthermore, since MBP-specific antibodies are also thought to contribute to the exacerbation of EAE and MS (Sun, 1993; Warren and Catz, 1993; Gerritse *et al.*, 1994) along with autoreactive MBP-specific T cells (Zamvil and Steinman, 1990; Zamvil *et al.*, 1986; Ando *et al.*, 1989; Lemire *et al.*, 1986; Hashim and Brewen, 1985), we analyzed MBP-specific antibody levels *in vivo* and MBP-specific B cell and T cell activation *in vitro* on IFNτ treated and control mice. Overall, these findings serve as the basis for understanding how type I IFNs exert therapeutic effects in autoimmune diseases.

Materials and Methods

IFNτ

The ovine IFNτ gene was expressed in *Pichia pastoris* using a synthetic gene construct, which was kindly provided by Dr. Gino Van Heeke, Ciba Pharmaceuticals, London, England (Heeke *et al.*, 1996). IFNτ was secreted into the medium and was purified by successive DEAE-cellulose and hydroxylapatite chromatography to electrophoretic homogeneity as determined by SDS-PAGE and silver staining analysis (Heeke *et al*, 1996). The purified protein had a specific activity of 2.9 to 4.4 x 10^7 U/mg protein as measured by antiviral activity using a standard viral microplaque reduction assay on MDBK (Pontzer *et al.*, 1991).

Induction of EAE

For induction of EAE, 300 μg of bovine MBP (MBP) were emulsified in complete Freund's adjuvant (CFA) containing 8 mg/ml H37Ra (*Mycobacterium tuberculosis*, Difco,

Detroit, MI) and injected into two sites at the base of the tails of NZW mice (Jackson Laboratory, Bar Harbor, Maine). On the day of immunization and 48 h later, 400 ng of pertussis toxin (List Biologicals, Campbell, CA) were also injected. For induction of EAE in SJL/J mice (Jackson Laboratory, Bar Harbor, Maine), the same protocol was used as described except mice were immunized again 7 days after the initial immunization. Mice were clinically examined daily for signs of EAE, and severity of disease was graded using the following scale: 1, loss of tail tone; 2, hind limb weakness, 3, paraparesis, 4, paraplegia; 5, moribund/death.

Administration of IFNτ

Mice were orally fed using feeding needles from Fisher Scientific (Norcross, GA) and injected intraperitoneally (i.p.) with 100 uL of 6 X 10^5 U total of IFNτ with PBS used as the vehicle for administration. Administration of IFNτ to SJL/J mice was started after the onset of EAE and was administered every 48 h thereafter. NZW mice were administered with IFNτ 48 h before, during, and 48 h after immunization with MBP.

Histological Evaluation

SJL/J Mice were perfused transcardially with 0.9% saline followed by Bouin's fixative solution. The vertebral columns were removed, and spinal cords were dissected out and post-fixed for an additional 48h in Bouin's fixative. Thereafter, the tissue was dehydrated and embedded in paraffin. Sections were cut at 7μm and mounted onto subbed slides. Cresyl violet staining was used to visualize inflammatory infiltrates.

For detection of microglia, SJL/J mice were first perfused with saline and 4% paraformaldehyde, and after removing the spinal cords and post fixing them for 1-2 h in 4% paraformaldehyde, they were then transferred to 30% sucrose solution for cryoprotection on at 4 °C. Spinal cord lumbar regions were embedded in OCT compound and 25 μm sections were cut on a cryostat and mounted onto subbed slides. Microglia were

detected by immunohistochemically staining for CD11b (CR3) with the monoclonal anti-mouse CD11b (CR3) antibody (SeroTec Inc., NC). A 1:300 dilution of monoclonal anti-mouse CD11b antibody was incubated with the sections overnight at 4 °C. Then, a 1:400 dilution of the secondary biotinylated anti-rat IgG antibody was incubated with the section for 1h at 25 °C followed by 1h incubations of avidin and horse radish peroxidase. Then, 3 3'-diaminobenzidine (DAB) (0.5 mg/ml) was applied to the sections for 10 minutes and finally washed with phosphate buffered saline (PBS), after which sections were counterstained with cresyl violet.

Proliferation assay and isolation of T cells and B cells

Spleen cells from IFNτ treated, PBS treated, or untreated MBP-immunized NZW or SJL/J mice (5.0×10^7 cells/well) were cocultured with IFNτ (10,000 - 30,000 U/ml), MBP (30 μg/ml), or media for 48 h. Spleen cells were washed, and B cells or T cells were isolated from each treated group using an immunoaffinity column from the cellect-plus mouse B cell kit or the cellect-plus mouse T cell kit (Biotex Laboratories, Alberta), respectively. B cell and T cell preparations were then incubated (5.0×10^5 cells/well) in RPMI 1640 media containing 5% FBS for 72 h. Some B cell and T cell cultures were incubated with 200 ng/ml of staphylococcol enterotoxin B (SEB) (Toxin Technology, FL), 50 μg/ml of lipopolysacharide (LPS) (Sigma chemical co., St. Louis, MO), or a combination of 20 ng/ml Phorbol 12-myristate 13-acetate (PMA) and 200 ng/ml ionomycin (Sigma Chemical Co., St. Louis, MO). The cultures were pulsed with [^{3}H]-thymidine (1.0 μCi/well)(Amersham, Indianapolis, IN) 18 h before harvesting onto filter paper discs using a cell harvester. Cell-associated radioactivity was quantified using a β-scintillation counter and activity is reported in CPM.

ELISA for MBP-Specific Antibodies

Bovine MBP was resuspended in binding buffer (0.1 M carbonate/bicarbonate, pH 9.6), adsorbed to the flat bottoms of plastic 96 well tissue culture wells overnight at 4 °C at a concentration of 600 ng/well, and evaporated to dryness. The plates were treated with blocking buffer, 5% powdered milk (Carnation) in PBS, for 2 h in order to block nonspecific binding and then washed 3 times with PBS containing 0.05% Tween 20. Various dilution of sera from NZW or SJL/J mice which were untreated, IFNτ-treated by i.p. injection, and IFNτ-treated by oral feeding, and PBS treated were added to the wells and incubated for 3h at room temperature. Binding was assessed with the secondary antibody, goat anti-mouse immunoglobulin (IgG) coupled to alkaline phosphatase. After the substrate solution, p-nitrophenyl phosphate (5mg/ml) was added and the reaction terminated with 3 N NaOH activity was monitored by color development at 405nm in an ELISA plate reader (Bio-Rad, Richmond, CA

Results

IFNτ Blocks Further Relapses into Paralysis of EAE-Afflicted Mice

The chronic form of EAE was induced in SJL/J mice with MBP, and these mice were treated with IFNτ (6×10^5 U) both orally and i.p. starting at the time of paralysis and at 48 h intervals thereafter. Both oral feeding and i.p. injection of IFNτ protected mice against further relapses of disease following a reduced primary relapse; protection against primary relapse was 80% for i.p. injected IFNτ, 65% via oral administration of IFNτ, and 17% for the PBS treated control group (Table 1). No further relapses were seen for the IFN-treated groups while 8 of 18 mice had secondary relapses in the PBS group during the treatment period. Thus oral administration and i.p. injection of IFNτ blocked further relapses into paralysis in mice afflicted with chronic EAE.

Table 1. Treatment of SJL mice with IFNτ after induction of EAE[a]

	Before treatment			During treatment (64 days)				
Treatment	Disease incidence	Mean day of onset	Mean severity	Primary relapsing incidence	Mean day of relapse	Mean severity	Secondary relapsing %Protected	incidence[b]
IFNτ-orally	20/20	17.9 ± 0.4	3.4	7/20	37.4 ± 7.8	2.9	65	0/20
IFNτ-i.p.	19/20	18.0 ± 0.5	3.5	4/20	40.3 ± 8.0	3.3	80	0/20
PBS	17/18	17.9 ± 0.7	3.2	15/18	34.0 ± 3.3	3.5	17	8/18

[a]SJL mice were immunized with MBP for induction of EAE. After the onset of disease (19 days after immunization), mice were injected intraperitoneally (i.p.) or orally fed with 6×10^5 U total of IFNτ every 48 h for 64 days. Mean severity and incidence of disease were recorded throughout the study for mice which relapsed into disease. Mice were clinically examined daily for signs of EAE, and severity of disease was graded using the following scale: 1, loss of tail tone; 2, hind limb weakness, 3, paraparesis, 4, paraplegia; 5, moribund/death. Protection of mice from relapse by oral administration ($p<0.005$) and i.p. injections ($p<0.001$) of IFNτ was statistically significant when compared to mice treated with PBS as determined by the χ^2 test.

[b]No further relapses into paralysis were seen in mice treated with IFNτ.

Reduction and Prevention of Lymphocytic Infiltrates in EAE Mice by IFNτ

Histology was performed to determine the extent of lymphocyte infiltration into the CNS of MBP immunized SJL/J mice afflicted with EAE before treatment and after treatment with IFNτ both i.p. and orally. Sections of the lumbar spinal cord were evaluated for lymphocyte infiltration after staining with cresyl violet. As shown in Figure 1A, perivascular accumulations of lymphocytes were present in the mouse spinal cord white matter prior to IFNτ treatment. Then, after 38 days in the absence of IFNτ, mice had lymphocytic infiltrates in their spinal cords (Figure 1B). In contrast, no lymphocytic infiltrates could be detected in mice treated with IFNτ by oral feeding (Figure 1C) or i.p. injection (Figure 1D) after 38 days. Thus, IFNτ reduced and prevented further lymphocytic infiltration into the CNS, demonstrating that the protective effect of IFNτ is associated with inhibition and reversal of lymphocyte infiltration of the CNS.

Deactivation of Microglia Following IFNτ Treatment

Spinal cord sections from SJL/J mice with EAE (Figure 2A) were marked by widespread and maximal microglial activation. This was evidenced by the fact that nearly all microglial cells within a given section had lost their ramified (branched) morphology and had transformed into brain macrophages appearing as enlarged rounded cells (Figure 2A). Following both oral and i.p. treatments with IFNτ, sections immunostained with anti-CD11b antibody showed only ramified microglia (Figure 2B, Figure 2C). Rounded brain macrophages were no longer detectable. The processes of ramified cells present after treatment did stain strongly with anti-CD11b antibody, and they were somewhat thicker than those of resting cells in CNS tissue from naive animals. Thus, it is likely that while microglia were de-activated by IFNτ treatment, they had not completely reverted back to the resting state, but had maintained low levels of activation.

Figure 1. Histological evaluation of EAE mice before and after treatment with PBS and IFNτ. SJL/J mice were immunized with MBP for induction of EAE and then treated with PBS or IFNτ, as per Table 1. SJL/J treatment groups evaluated included mice before any treatment (A), PBS treatment (B), IFNτ treatment by oral feeding (a normal blood vessel with no lymphocyte infiltration is presented for comparison) (C), and IFNτ treatment by i.p. injection (D). Histology was evaluated after the induction of EAE 19 days after immunization in A, and 38 days after the initiation of the treatments (57 days after immunization) in B-D. Two mice per group from separate experiments were examined. Sections of spinal cord were stained with cresyl violet and presented at a final magnification of 400X.

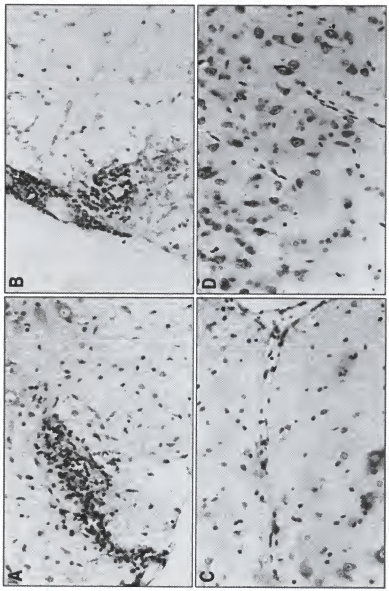
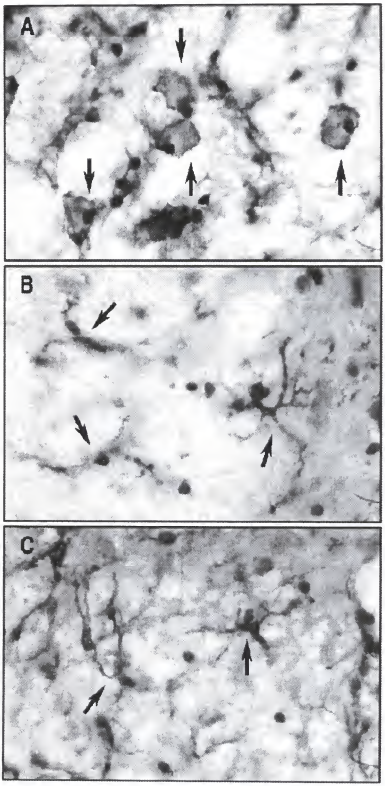

A
B
C
D

Figure 2. IFNτ causes de-activation of microglia. Spinal cord section from PBS treated SJL/J mouse with EAE shows fully activated microglial cells which exist as rounded brain macrophages (arrows in A). Oral feeding (B) and i.p. injection (C) of IFNτ caused microglia to assume a process-bearing (arrows) morphology indicating a reversion to the resting state. Sections were taken 38 days after initiation of the treatments (57 days after immunization), and two mice per group from separate experiments were examined. Sections of spinal cord were immunohistochemically stained for microglia and counterstained with cresyl violet and are presented at a final magnification of 1000X. Mice were from the PBS and oral and i.p. IFNτ treated groups from Table 1.


A
B
C

IFNτ Treated Mice Have a Lower Antibody Level Against MBP than Control Mice

During the course of IFNτ treatment of SJL/J mice for chronic relapsing EAE, mice were bled and sera were examined for the presence of MBP-specific antibodies. As shown in Figure 3, mice that received i.p. injections of IFNτ had the lowest anti-MBP antibody level followed by the orally fed mice. Untreated and PBS treated mice had the highest anti-MBP antibody levels. Similarly, in the acute form of EAE, which was induced in NZW mice, anti-MBP antibody levels were also lower in the IFNτ treated group than in the PBS treated group (Figure 4). Thus, IFNτ inhibition of antibody production against MBP may be a contributing mechanism by which IFNτ prevents development or inhibits further relapses of EAE in the acute and chronic forms of the disease, respectively.

IFNτ Inhibited MBP-Specific B Cell Proliferation

Related to antibody production, we addressed the question of whether IFNτ had any effect on B cell activation in response to MBP in culture. Splenocytes obtained from SJL/J mice that had been immunized with MBP for induction of chronic EAE were incubated with media or IFNτ in the presence or absence of MBP for 48 hr. B cells were isolated using an immunity affinity column and then incubated with media alone for 72 h, after which proliferation was measured. As shown in Figure 5, B cells isolated from IFNτ-treated SJL/J splenocyte cultures did not proliferate in the presence of MBP, while the media treated B cells did respond to MBP significantly. B cells that were isolated with the immunoaffinity column did not proliferate in response to the T cell superantigen staphylococcal enterotoxin B (SEB), but did proliferate in the presence of lipopolysacharide (LPS), a polyclonal B cell stimulator (Figure 5 inset). Thus, the proliferation seen in Figure 5 is from B cells and not from T cell contamination. Furthermore, splenocytes obtained from IFNτ and PBS-treated NZW mice, which developed the acute form of EAE, were incubated in media alone or with IFNτ in the presence or absence of MBP for 48 h, after which B cells were isolated and incubated with media alone for 72 h and proliferation

Figure 3. Inhibition of anti-MBP antibody production in the chronic form of EAE by IFNτ. Sera from SJL/J mice were obtained before MBP immunization (normal mouse serum (NMS)), at the time of paralysis (before treatment), and 38 days after the initiation of treatment with PBS and IFNτ i.p. and orally as per Table 1. Direct ELISA was performed to detect anti-MBP antibodies. Four mice per group were used, and average absorbance is shown. Statistical significance for the inhibition of anti-MBP antibody production was shown by Student's t-test at the 100 dilution factor for oral IFNτ treatment ($p<0.01$) and i.p. IFNτ treatment ($p<0.001$) as compared to the PBS treatment.

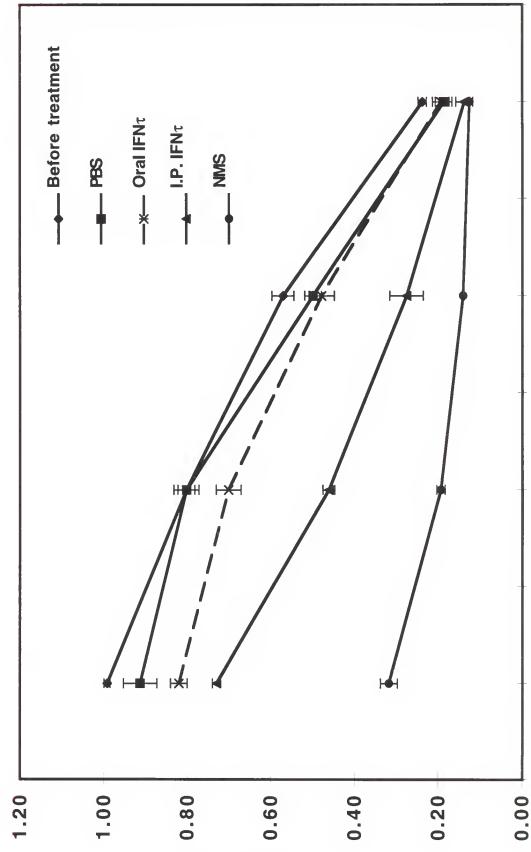

Before treatment
PBS
Oral IFNτ
I.P. IFNτ
NMS
Absorbance
1.20
1.00
0.80
0.60
0.40
0.20
0.00
10
100
1000
10000
Dilution factor

Figure 4. Inhibition of anti-MBP antibody production in the acute form of EAE by IFNτ. NZW mice were immunized with MBP for induction of acute EAE. Mice were given IFNτ (6×10^5 U/treatment time) or PBS by i.p. injection at the time of, 48 h before, and 48 h after immunization. Sera were obtained from mice before immunization with MBP (normal mouse serum (NMS)), and three weeks after immunization from the PBS and IFNτ treated groups. Direct ELISA was performed to detect anti-MBP antibodies. Pooled sera from four mice per group were used and average absorbance is shown. Statistical significance for the inhibition of anti-MBP antibody production was shown by Student's t-test at 1000 and 3000 dilution factors between IFNτ and PBS treatments ($p<0.001$).

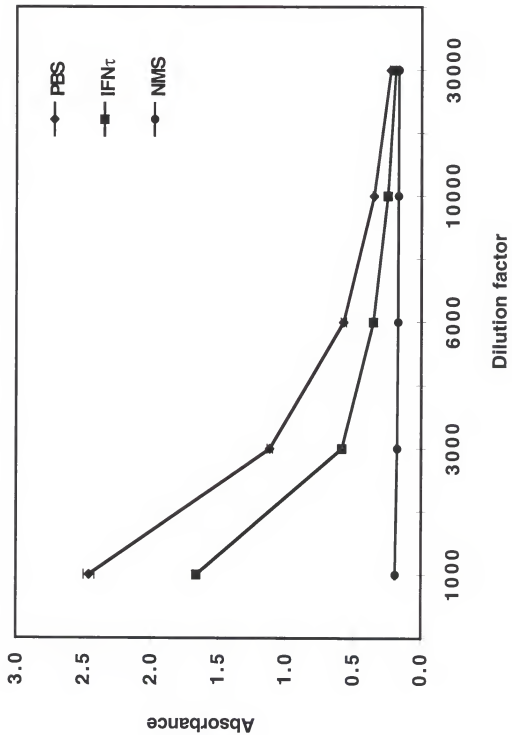
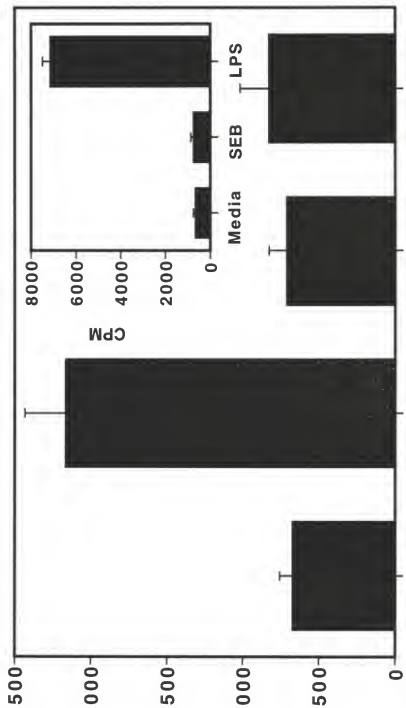

PBS
IFNτ
NMS
Absorbance
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1000
3000
6000
10000
30000
Dilution factor

Figure 5. IFNτ inhibits MBP-specific proliferation of B cells from chronic EAE mice. SJL/J mice were immunized with MBP for induction of EAE. After three weeks, spleen cells were taken and treated with IFNτ (3.0×10^4 U/ml) or media in presence or absence of MBP for 48 h in culture. B cells were isolated and reincubated (5×10^5 cell/well) in media alone for another 48 h. B cells from media treated splenocytes were also incubated with 200 ng/ml SEB or 50 μg/ml LPS for negative and positive controls of B cell isolation, respectively (figure inset). All cultures were pulsed with tritiated thymidine, and cell-associated radioactivity was quantified 18 h later, and data from one of three respective experiment are presented as mean CPM of quadruplicate wells ± SD. IFNτ's inhibition of MBP-specific B cell proliferation was statistically significant as compared to the MBP-specific B cell proliferation of MBP sensitized media treated cells as shown by the Student's t-test ($p<0.001$).


2500
2000
1500
1000
500
0
CPM
Media
Media+MBP
IFNτ
IFNτ+MBP
Treatment
8000
6000
4000
2000
0
CPM
Media
SEB
LPS

measured. As shown in Figure 6, EAE B cells isolated from MBP-treated splenocytes that were taken from a PBS-treated NZW mouse had a stimulation index greater than 11 relative to media control, while EAE B cells isolated from IFNτ-treated splenocytes did not proliferate in response to MBP. Furthermore, EAE B cells isolated from MBP-treated splenocytes that were taken from an IFNτ-treated NZW mouse did not proliferate either. Thus, IFNτ can inhibit activation of B cells, taken from mice having either acute or chronic EAE, in response to MBP both *in vivo* and *in vitro*, consistent with reduction of antibody production to MBP in IFNτ treated EAE mice.

IFNτ Inhibited MBP-Specific T Cell Proliferation

Previously, we have shown that IFNτ inhibits MBP-specific splenocyte proliferation (4) by inducing CD4 T cell to produce IL-10 and TGFβ which act synergistically to inhibit EAE splenocyte activation (Mujtaba *et al.*, 1997). Here we determine the effect of IFNτ on proliferation of T cells isolated from MBP-sensitized SJL/J mouse splenocytes stimulated with MBP and treated with IFNτ or media in culture. At both IFNτ concentrations, MBP activation of MBP-specific T cell were inhibited, and at 30,000 U/ml, IFNτ reduced levels of proliferation to less than 50% of that observed in response to MBP alone (Figure 7). The T cells that were isolated with the immunoaffinity columns did not proliferate in response to LPS, but did proliferate in presence of PMA and ionomycin, which are T cell activators when used in combination (Figure 7 inset). Therefore, the proliferation seen in Figure 7 is from T cells and not from B cell contamination. Thus, IFNτ inhibits the activation of MBP-specific effector T cells of chronic EAE mice.

Figure 6. IFNτ inhibits MBP-specific proliferation of B cells from acute EAE mice. NZW mice were immunized with MBP for induction of acute EAE. Mice were given IFNτ (6×10^5 U/treatment time) or PBS by i.p. injection at the time of, 48 h before, and 48 h after immunization. After three weeks, splenocytes were taken from the PBS and IFNτ groups and treated with IFNτ (3.0×10^4 U/ml) or media in the presence or absence of MBP for 48 h in culture. B cells were then isolated and reincubated (5×10^5 cell/well) in media alone for another 48 h after which the cultures were pulsed with tritiated thymidine. Cell-associated radioactivity was quantified 18 h later using a β-scintillation counter, and data from one of three respective experiment are presented as mean CPM of quadruplicate wells ± SD. Inhibition of B cell proliferation with both *in vitro* and *in vivo* IFNτ treatments were statistically significant when compared to the MBP treatment alone as shown by Student's t-test ($p<0.001$).

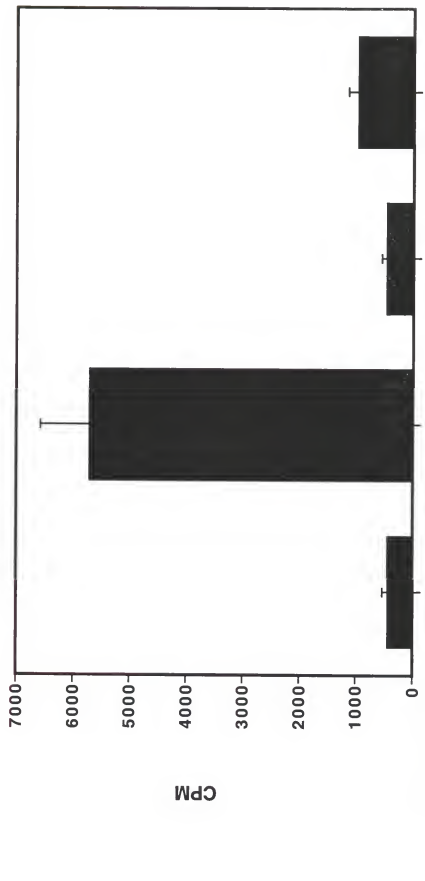
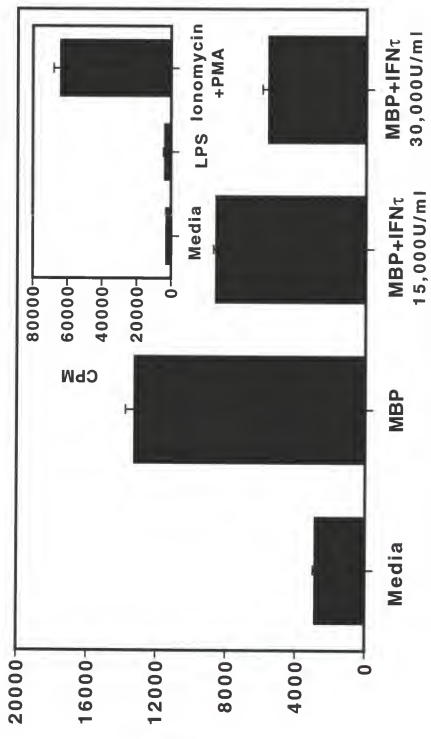

CPM
7000
6000
5000
4000
3000
2000
1000
0
EAE mice treated with: PBS PBS PBS IFNτ
In vitro treatment for B cell proliferation: Media Media + MBP IFNτ + MBP Media + MBP

Figure 7. IFNτ inhibits MBP-specific T cell proliferation. SJL/J mice were immunized with MBP for induction of EAE. After three weeks, spleen cells were taken and treated with IFNτ (3.0×10^4 U/ml) or media in the presence or absence of MBP for 48 h in culture. T cells were isolated and reincubated (5×10^5 cell/well) in media alone for another 48 h. T cells from media treated splenocytes were also incubated with 50 μg/ml LPS or with 20 ng/ml PMA and 200 ng/ml ionomycin for negative and positive controls of T cell isolation, respectively (figure inset). All cultures were pulsed with tritiated thymidine, and cell-associated radioactivity was quantified 18 h later, and data from one of three respective experiment are presented as mean CPM of quadruplicate wells ± SD. MBP-specific T cell proliferation of both IFNτ concentrations were statistically significant from the MBP-specific T cell proliferation of MBP treated cells alone as shown by the Student's t-test ($p<0.001$).


CPM
20000
16000
12000
8000
4000
0
Media
MBP
MBP+IFNτ
15,000U/ml
MBP+IFNτ
30,000U/ml
Treatment
CPM
80000
60000
40000
20000
0
Media
LPS
Ionomycin
+PMA

Discussion

The findings reported here clearly show that ongoing chronic, relapsing EAE in SJL/J mice can be significantly alleviated by either parenteral or oral administration of IFNτ on a continuous basis. Although some mice in both IFNτ treatment groups had primary relapsing incidence of paralysis during treatment, further relapses were not detected unlike the PBS treated group. Parenteral administration of IFNτ was slightly more effective in alleviating chronic EAE than oral administration in that only 4 of 20 mice had relapses while 7 of 20 mice had primary relapses in the orally treated group. Furthermore, untreated EAE mice showed lymphocyte infiltration of the CNS and activation of microglia, whereas, IFNτ treatment of mice with active EAE reversed these cellular effects. This suggests that IFNτ treatment results in relief or permanent remission of the observed EAE symptoms.

Although considerable attention is usually given to T-helper cell-mediated events in EAE, we show that anti-MBP antibody and MBP-specific B cell effects, like the T cell effects, are inhibited by IFNτ in both chronic and acute forms of EAE. MBP antibody production in EAE mice was significantly inhibited by IFNτ, with i.p. administration of IFNτ more effective than oral, and MBP induced proliferation of sensitized B cells was also blocked. IFNτ inhibition of B cell proliferation probably plays a central role in inhibition of anti-MBP antibody production. We have shown that IFNτ and other type I IFNs induce terminal differentiation of Daudi B cells to plasma cells (Subramaniam *et al.*, 1998). Thus, IFNτ inhibition of B cell clonal expansion by induction of terminal differentiation, which results in an overall lower plasma cell number, probably plays a central role in IFNτ inhibition of production of antibodies to MBP in EAE mice. Overall, we have demonstrated that IFNτ is an effective therapy for ongoing EAE and as such should have potential for treatment of MS in humans.

CHAPTER 3
CD4 T SUPPRESSOR CELLS MEDIATE IFNτ PROTECTION AGAINST EAE

Introduction

Previously, we showed that interferon tau (IFNτ) blocks the development of experimental allergic encephalomyelitis (EAE) in mice without associated toxicity; however the mechanism of such action has not been fully elucidated (Soos *et al.*, 1995a). EAE is a murine model useful for studying the demyelinating disease multiple sclerosis (MS) (Zamvil and Steinman, 1990). Myelin basic protein (MBP) has been shown to be one of the primary central nervous system antigens responsible for induction of autoimmunity in the EAE model. Upon immunization with MBP, mice develop clinically observable tail and limb paralysis due to lymphocyte infiltration into the central nervous system accompanied by acute demyelination (Zamvil and Steinman, 1990).

The type I IFNs, α and β, have previously been shown to induce suppressor cells that block *in vitro* antibody production (Johnson and Blalock, 1980). Further, when type I IFN-induced suppressor cells were cultured *in vitro*, they were shown to produce a soluble factor that mediated immunosuppression. Past studies by others suggested that "classic" T suppressor cells bear the CD8 phenotype. Here we demonstrate that IFNτ-induced suppressor cells bear the CD4 phenotype, and these cells mediate the amelioration of EAE by IFNτ. In addition, IFNτ-induced suppressor cell function occurs via a mechanism similar to that originally observed for type I IFNα and β inhibition of antibody production *in vitro*. A suppressor mechanism shared by the type I IFNs is the induction of soluble suppressor factors, which we demonstrate in this study. These findings serve as the basis

for understanding how type I IFNs exert therapeutic effects in autoimmune diseases such as MS.

Materials and Methods

IFNs

The ovine IFNτ (IFNτ) gene was expressed in *Pichia pastoris* using a synthetic gene construct, which was kindly provided by Dr. Gino Van Heeke, Ciba Pharmaceuticals, London, England (Heeke *et al.*, 1996). IFNτ was secreted into the medium and was purified by successive DEAE-cellulose and hydroxylapatite chromatography to electrophoretic homogeneity as determined by SDS-PAGE and silver staining analysis. The purified protein had a specific activity of 2.9 to 4.4 x 10^7 U/mg protein as measured by antiviral activity using a standard viral microplaque reduction assay on MDBK cells (Pontzer *et al*, 1991). MuIFNβ (specific activity 4.1 x 10^7 U/mg) was obtained from Lee Biomolecular (San Diego, CA).

Antibodies and Cytokines

Monoclonal rat anti-mouse IL-10, recombinant mouse IL-10, and monoclonal mouse anti-TGF-β_1,-β_2,-β_3 were obtained from Genzyme, Cambridge, MA. Ultrapure natural human TGFβ_1, which shows cross-reactivity in most mammalian cell types, was also obtained from Genzyme. A 1:10 dilution of HL100, a specific monoclonal antibody for IFNτ, was used to neutralize 5000 U/ml of IFNτ prior to usage. The HL100 monoclonal antibody was kindly provided by Dr. Carol Pontzer, University of Maryland, College Park, MD. All antibodies and cytokines were used in proliferation assays described below.

Interferon Induction of Suppressor Cells

Suppressor cells were induced both *in vitro* and *in vivo*. For *in vitro* induction, NZW mouse spleen cells (5.0×10^7/ml) were incubated with 5000 U/ml of IFNτ for 24 h at 37°C, after which the cells were washed twice prior to use. *In vivo* induction of suppressor cells in naive NZW mice involved administration of a single dose of IFNτ (10^5 U) either intraperitoneally (i.p.) or by oral feeding with PBS used as the vehicle for administration. After 24 h, mice were sacrificed and the spleens removed. Spleen cells were washed and resuspended in RPMI 1640 medium supplemented with 2% fetal bovine serum and used as described below.

Induction of EAE

For induction of EAE, 300 μg of bovine MBP (MBP) were emulsified in complete Freund's adjuvant (CFA) containing 8 mg/ml H37Ra (*Mycobacterium tuberculosis*, Difco, Detroit, MI) and injected into two sites at the base of the tails of NZW mice. On the day of immunization and 48 h later, 400 ng of pertussis toxin (List Biologicals, Campbell, CA) were also injected. Mice were clinically examined daily for signs of EAE, and severity of disease was graded using the following scale: 1, loss of tail tone; 2, hind limb weakness, 3, paraparesis, 4, paraplegia; 5, moribund/death.

Adoptive Transfer of Suppressor Cells

Suppressor cells were induced *in vitro* with IFNτ as described above and resuspended in phosphate buffered saline (PBS). NZW mice were injected intraperitoneally with 100 μl of PBS containing 5×10^6 suppressor cells 48 h before, on the day of, and 48 h after immunization with MBP for induction of EAE. Mice were examined daily for signs of EAE, and the severity of disease was graded as noted above.

CD4 T Cell Isolation and Depletion

CD4 T cells effects were examined using both positive and negative CD4 T cell selection processes. The Cellectplus mouse CD4 kit (Biotex Laboratories, Inc., Alberta, Canada), an immuno affinity column, was used to isolate CD4 cells from NZW mouse spleen lymphocyte cultures treated with media or IFNτ. Depletion of CD4 T cells from mouse spleen lymphocyte cultures treated with IFNτ or media was carried out using rat anti-mouse L3/T4 CD4 monoclonal antibody (Biosource International, Camarillo, CA) and Low-Toxic-M rabbit complement (Accurate Chemical and Scientific Corporation, Westbury, NY). Lymphocytes from NZW mouse spleen were resuspended at 10^7 cells/ml in RPMI 1640 medium and incubated with 1:10 dilution of anti-mouse L3/T4 CD4 antibody for 1 h at 4° C. Cells were then centrifuged and resuspended in 1:10 dilution of rabbit complement in RPMI 1640 medium for 1 h at 37°C. The cultures were washed and used for further experimentation.

Production of Suppressor Factor

Suppressor cells were generated *in vitro* by incubating spleen cells with 5000 U/ml of IFNτ for 24 h at 37°C as described above. Cells were then washed and resuspended at 10^8 cells/ml in fresh culture medium. After incubating for an additional 2 h at 37°C, clarified supernatants were collected and tested for suppressor activity.

Proliferation Assay

Spleen cells from MBP-immunized NZW mice (2.5 - 5.0 x 10^5 cells/well) were cocultured with IFNτ- or IFNβ-induced suppressor cells (1.0-5.0 x 10^5/well), suppressor cell supernatants, or IL-10 and TGFβ in the presence of 30 or 100 μg/ml of MBP. Suppressor cell supernatants were also pretreated for 2 h with either anti-IL10 antibody (25 μg/ml) or anti-TGFβ antibody (25 μg/ml) and then cultured with MBP-specific cells in the presence of MBP. Cultures were incubated for 96 h at 37° C. The cultures were then

pulsed with [^{3}H]-thymidine (1.0 μCi/well; Amersham, Indianapolis, IN) 18 h before harvesting onto filter paper discs using a cell harvester. Cell-associated radioactivity was quantified using a β-scintillation counter. Stimulation index was determined by dividing experimental CPM by control (unstimulated) CPM.

Results

IFNτ-Treated Spleen Cells Inhibit MBP-Specific T Cell Proliferation

We first addressed the question of whether IFNτ can suppress MBP-specific T cell proliferation by induction of suppressor cells in NZW mouse spleen cells. Spleen cells were treated with IFNτ in tissue culture or were obtained from mice injected intraperitoneally (i.p.) with IFNτ, or from mice treated orally with IFNτ. IFNτ-treated spleen cells from all three sources inhibited MBP induced proliferation of spleen cells from EAE mice by as much as 80% relative to the control response (Figure 8). Similar to type I IFN induction of suppressor cells for antibody production (Johnson and Blalock, 1980), IFNτ suppressed MBP-specific immune response via induction of suppressor cells.

IFNτ Induction of Suppressor Cells are Dose-Dependent

IFNτ and IFNβ were compared at various concentrations for induction of suppressor cells in spleen cell cultures for inhibition of MBP stimulation of sensitized cells from EAE mice (Figure 9). For both IFNs, the induction of suppressor cells was dose dependent. IFNβ was slightly more effective at induction of suppressor cells, but the slopes of the dose response curves for the two IFNs were similar. Thus, Type I IFN induction of suppressor cells is dose-dependent.

Figure 8. IFNτ-treated spleen cells inhibit MBP-specific T cell proliferation. Suppressor cells were induced with IFNτ both *in vitro* and *in vivo* . IFNτ-treated spleen cells were washed and cocultured at 1.0 x 10^5 cells/well with MBP-specific mouse spleen cells (2.5 x 10^5 cells/well) and MBP protein at 100 μg/ml for 96 h. Media-treated spleen cells served as controls. Data from one of three representative experiments are presented as mean CPM ± S.D. of quadruplicate cultures. Proliferation was measured by [^{3}H]-thymidine incorporation. IFNτ suppressor cells induced by all three methods showed significant suppression by χ^2 test with $p < 0.001$ relative to MBP-stimulated controls.

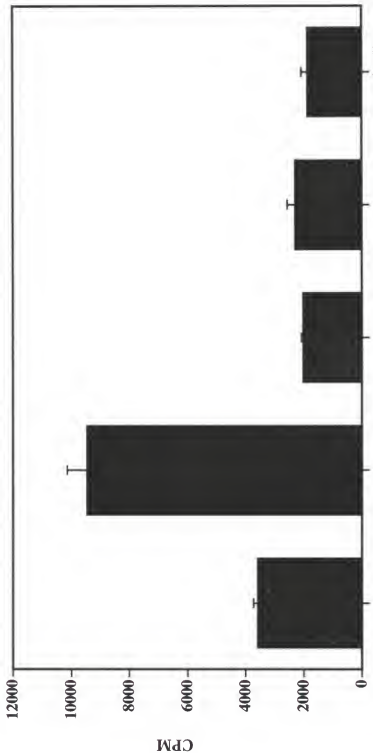
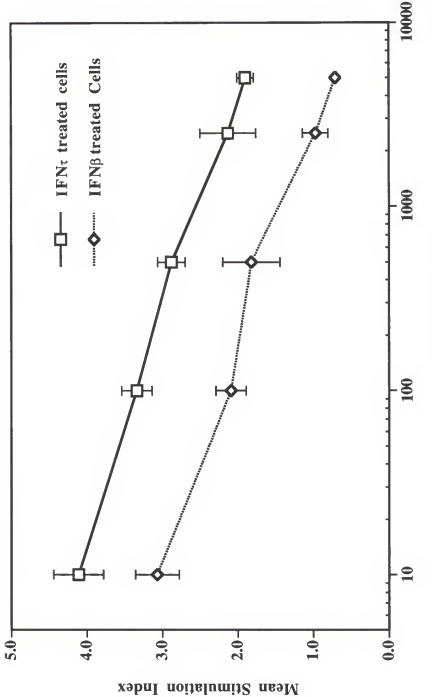

12000
10000
8000
6000
4000
2000
0
CPM
None
None
Tissue culture
Intraperitoneal
Oral feeding
MBP: –
+
+
+
+
IFNγ treatment

Figure 9. IFNτ induction of suppressor cells is dose-dependent. NZW spleen cells (5×10^7 cell/ml) were treated with IFNτ and IFNβ at various concentrations for 24 h *in vitro* . IFN-treated cells (5×10^5 cells/well) were then cocultured with MBP-specific cells (2.5×10^5 cells/well) in the presence of 100 μg/ml of MBP protein. Proliferation was measured by [^{3}H]-thymidine incorporation. Data from one of three representative experiments are presented as mean stimulation index ± S.D. of quadruplicate cultures. Coculture of MBP-specific cells and media-treated cells had a stimulation index of 7.5 ± 3.3. The CPM for unstimulated cells were 405 ± 97.


Mean Stimulation Index
5.0
4.0
3.0
2.0
1.0
0.0
10
100
1000
10000
IFN Concentration (U/ml)
IFNτ treated cells
IFNβ treated Cells

IFNτ Suppressor Cells Protect Mice Against EAE

Adoptive transfer of IFNτ-induced suppressor cells to NZW mice immunized with bovine MBP was carried out in order to determine if the suppressor cells protected the mice from development of EAE. NZW mice have previously been shown to be susceptible to development of EAE after immunization with either rat MBP (Zamvil *et al.*, 1994; Kumar *et al.*, 1994) or bovine MBP (J. Schiffenbauer, unpublished observation). Others have shown the transfer of peripheral cells from orally administered IFN donor mice to recipient mice causes suppression of white blood cells (Fleischmann *et al.*, 1992). Suppressor cells induced in culture with IFNτ were injected i.p. 48 h before, at the time of, and 48 h after immunization of mice with MBP. Suppressor cell-treated mice showed delayed onset of EAE (34.3 days) compared to untreated controls (19.6 days), and the incidence of EAE was 3 of 5 with lower severity of disease for suppressor cell-treated mice compared to 5 of 5 with higher severity of disease for untreated mice (Figure 10). Thus, adoptive transfer of IFNτ induced suppressor cells significantly protected mice against EAE.

IFNτ-Induced Suppressor Cells are CD4 T Cells

We next determined the phenotype of the suppressor cells by using antibody affinity columns to purify CD4 T cells and using specific CD4 antibody and complement to deplete CD4 T cells from IFNτ-treated cultures (Figure 11). CD4 T cells purified from an IFNτ-treated spleen cell preparation inhibited MBP-specific T cell responses by almost 50%, while non-CD4 T cultures from IFNτ-treated spleen cells were without effect. The non-CD4 T cell preparations consisted of CD8 T cells, macrophages, and other cells. Thus, the suppressor cell appears to be a CD4 T cell.

Figure 10. IFNτ-induced suppressor cells can delay the onset of EAE in mice. NZW mice were injected i.p. with IFNτ-treated whole spleen cells ($5x10^6$) 48 h before, on the day of, and 48 h after immunization with bovine MBP for induction of EAE. Mice were followed daily for signs of EAE, and mean severity of paralysis for each group was graded based on the scale mentioned in the materials and methods. Control mice had a average severity of 2.8, while adoptive transferred mice had a severity of 2.0. Mean day of onset of paralysis for the control and suppressor cell-treated mice were 19.6 ± 2.6 and 34.3 ± 2.3 days, respectively. The delay of onset of paralysis was statistically significant as shown by student's t-test ($p < 0.001$).

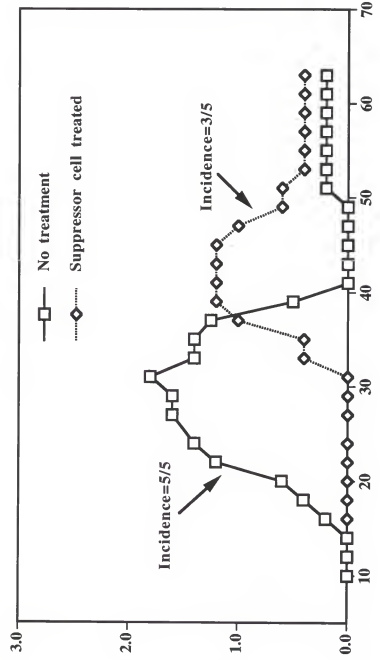
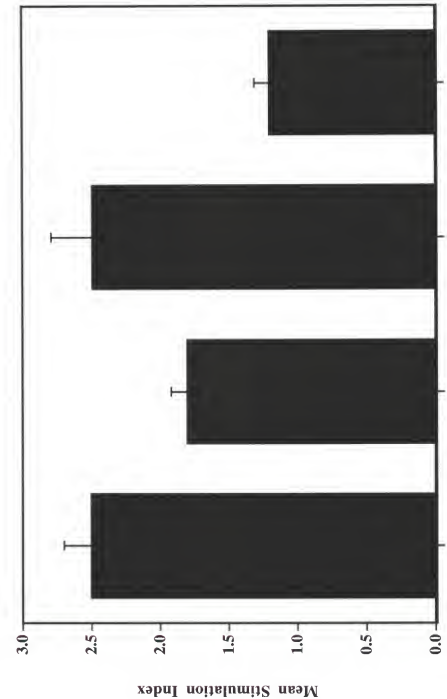

No treatment
Suppressor cell treated
Incidence=5/5
Incidence=3/5
Mean Severity
3.0
2.0
1.0
0.0
10
20
30
40
50
60
70
Days after treatment

Figure 11. IFNτ-induced suppressor cells are CD4 T cells. NZW mouse spleen cells were treated with media or IFNτ for 24 h *in vitro*. CD4 T cells were isolated from IFNτ-treated cell cultures using an immunoaffinity column. CD4 T cells were depleted from a second set of IFNτ-treated cells using anti-CD4 antibody plus complement. Whole and fractionated spleen cells (2.5×10^5) were cocultured with MBP-specific cells (2.5×10^5 cell/well) in the presence of 30 μg/ml of MBP. Proliferation was measured by [^{3}H]-thymidine incorporation. Data from from one of two representative experiments are presented as mean stimulation index ± S.D. of quadruplicate cultures. Cocultures of MBP-specific cells and fractionated media-treated cells had similar stimulation indices as those with whole unfractionated cells. Statistical significance by χ^2 test for CD4 T cell suppression was $p<0.001$ relative to the media treated spleen cell control. The CPM for unstimulated cells were 1003 ± 183.


Mean Stimulation Index
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Media treated spleen cells
IFNγ treated spleen cells
IFNγ treated Non-CD4 T cells
IFNγ treated CD4 T cells

Suppressor Cells Produce Soluble Suppressor Factor(s)

We previously showed that type I IFN-treated cells produced a suppressor factor for production of antibody to sheep red blood cells (Johnson and Blalock, 1980). IFNτ-treated cells were thus examined for production of a suppressor factor. As shown in Figure 12A, supernatants from IFNτ-treated spleen cells that had been incubated for 2 h at 37° C inhibited MBP-specific T cell responses. Inhibitory supernatants were not produced by cells treated with IFNτ that had been neutralized with specific antibody prior to treatment of cells. Further, the antibodies did not inhibit suppressor cell activity when added to cells after treatment with IFNτ. Consistent with the CD4 T cell phenotype of the suppressor cell, supernatants from IFNτ-treated CD4 T cells suppressed the MBP-specific responses (Figure 12B). Thus, the IFNτ-induced CD4 suppressor T cell produces soluble suppressor factor(s).

IFNτ-Induced Suppressor Cells Produce IL-10 and TGFβ

We next characterized the suppressor factors that IFNτ induced in spleen cells using antibodies to IL-10 and TGFβ. As shown in Figure 13A, both monoclonal anti-IL-10 and monoclonal anti-TGFβ antibodies blocked the suppressive activity of the suppressor cells on MBP-specific T cell responses. Similarly, both anti-IL-10 and anti-TGFβ antibodies neutralized the suppressive activity of supernatants from IFNτ-induced suppressor cells on the MBP-specific T cell responses (Figure 13B). Not unexpectedly, the antibodies to IL-10 and TGFβ combined showed complete recovery as did each antibody showed separately (data not shown). Also, addition of the corresponding cytokines in excess reversed the blockage of suppression by the antibodies. The control monoclonal anti-IFNτ antibody had no effect on the suppressor activity of the suppressor cells or their supernatant. Thus, both anti-IL-10 and anti-TGFβ antibodies restored the MBP-induced response to that of the

Figure 12. IFNτ-induced CD4 T suppressor cells produce soluble suppressor factor(s). NZW mouse spleen cells were treated with media or with 5000 U/ml IFNτ in the presence and absence of neutralizing antibody to IFNτ (mAb HL-100) (panel A). CD4 T cells were isolated from media and IFNτ-treated cultures (panel B). After washing, the cells were incubated for 2 h in media, and supernatants were collected. Supernatants were incubated with MBP-specific mouse spleen cells (5.0×10^5 cells/well) in the presence of 30 μg/ml of MBP protein. Proliferation was measured by [^{3}H]-thymidine incorporation. Data from one of two representative experiments are presented as mean stimulation index ± S.D. of quadruplicate cultures. In panel A, statistical significance by χ^2 test for suppressive activity of supernatants from cells pretreated with IFNτ was $p < 0.001$. In panel B, statistical significance by χ^2 test for suppressive activity of supernatants from CD4 T cells pretreated with IFNτ was $p < 0.001$. The CPM for the unstimulated cells were 138 ± 36 in panel A and 262 ± 52 in panel B.

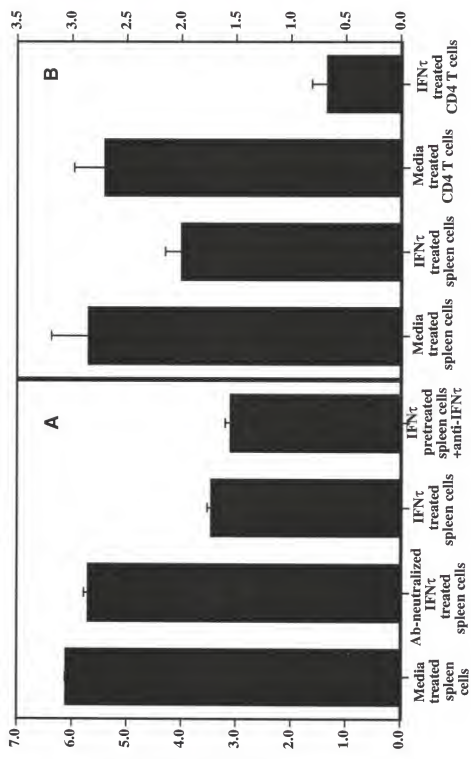
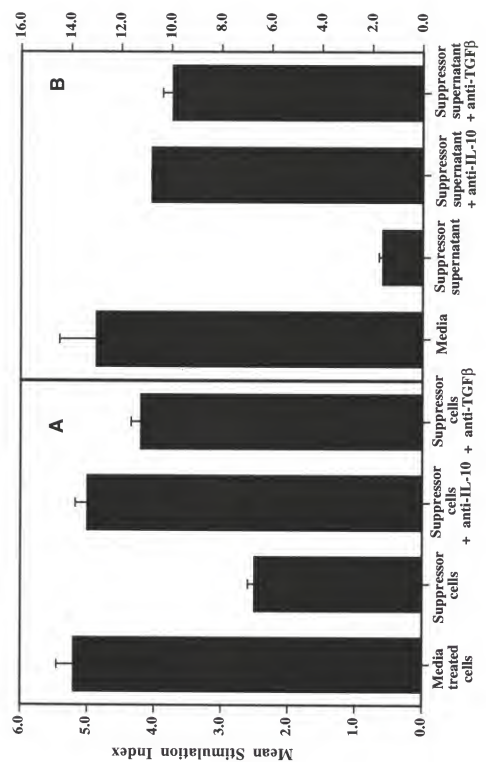

Mean Stimulation Index
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
A
B
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Media treated spleen cells
Ab-neutralized IFNτ treated spleen cells
IFNτ treated spleen cells
IFNτ pretreated spleen cells +anti-IFNτ
Media treated spleen cells
IFNτ treated spleen cells
Media treated CD4 T cells
IFNτ treated CD4 T cells
Source of supernatants

Figure 13. Blockage of IFNτ-induced suppressor cell and suppressor supernatant effects on MBP stimulation of sensitized EAE mouse spleen cells with monoclonal antibodies to IL-10 and TGFβ. Media-treated spleen cells and suppressor spleen cells (3.0×10^5 cells/well) induced *in vitro* with IFNτ (panel A) and their supernatants (panel B) were each cocultured with MBP-sensitized spleen cells (5×10^5 cells/well) in the presence of 30 μg/ml of MBP. Anti-IL-10 (25 μg/ml) and anti-TGFβ (25 μg/ml) antibodies were added at initiation of cultures. Proliferation was assessed after 96 h by [^{3}H]-thymidine incorporation. Data from one of two representative experiment are presented as mean stimulation index ± S.D. of quadruplicate cultures. The effects of anti-IL-10 and anti-TGFβ antibodies on media treated cells were similar to those of media controls shown. In panel A, blockage of the suppressive activity of the suppressor cells by both antibodies was statistically significant ($p < 0.001$) as determined by the χ^2 test. In panel B, blockage of the suppressive activity of the suppressor cell supernatant by both antibodies was statistically significant ($p < 0.001$) as determined by the χ^2 test. The CPM for the unstimulated cells were 4843 ± 130 in panel A and 2716 ± 18 in panel B.


Mean Stimulation Index
6.0
5.0
4.0
3.0
2.0
1.0
0.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
A
B
Media treated cells
Suppressor cells
Suppressor cells + anti-IL-10 + anti-TGFβ
Suppressor cells
Media
Suppressor supernatant
Suppressor supernatant + anti-IL-10
Suppressor supernatant + anti-TGFβ

control, which suggests a synergistic interaction between IL-10 and TGFβ in inducing suppression.

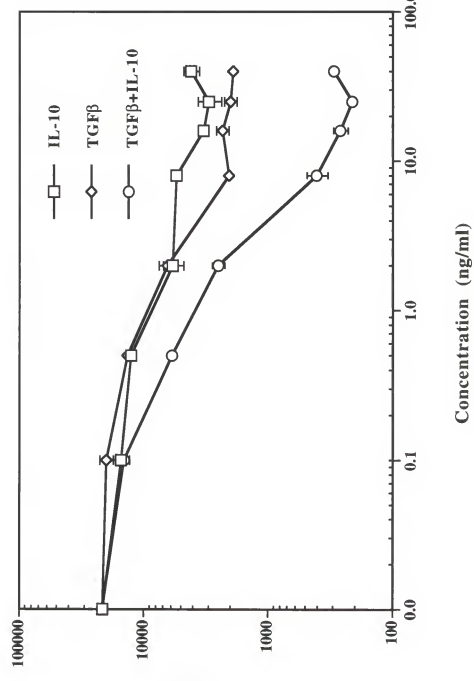
IL-10 and TGFβ Act Synergistically to Inhibit MBP-Specific T Cell Responses

We next evaluated the possible synergistic interaction between IL-10 and TGFβ on suppression of MBP-induced mouse spleen cell proliferation. As shown in Figure 14, both IL-10 and TGFβ suppressed MBP-specific T cell responses individually, but IL-10 and TGFβ together enhanced the suppression of MBP-sensitized spleen cell proliferation in response to MBP. Both IL-10 and TGFβ at a concentration of 8 ng/ml each, greatly reduced MBP-specific responses, compared to that obtained at 16 ng/ml of each factor. Thus, the combined effects of IL-10 and TGFβ are apparently not additive. These data suggest that IL-10 and TGFβ act synergistically at certain concentrations to inhibit MBP-induced EAE spleen cell proliferation.

Discussion

Data presented here demonstrate that IFNτ induces CD4 T cells to become suppressor cells in NZW mice by oral administration or intraperitoneal injection of IFNτ, and by treatment of mouse spleen cells with IFNτ in tissue culture. The suppressor cells inhibit MBP stimulation of spleen cells from MBP-immunized mice, and protect mice against induction of EAE. Also, the CD4 T suppressor cells produce both IL-10 and TGFβ, which act synergistically to inhibit MBP-specific T cell proliferation. Induction of suppressor cells can be blocked by pretreatment but not posttreatment of IFNτ with neutralizing antibodies, thus establishing that induction of suppressor cells is specific for IFNτ, but is not itself IFNτ. Therefore, IFNτ inhibition of EAE appears to occur via induction of suppressor cells and their suppressor factors such as IL-10 and TGFβ.

Figure 14. IL-10 and TGFβ act synergistically to inhibit MBP-specific T cell responses. Varying concentrations of IL-10 and TGFβ individually and together were preincubated with MBP-sensitized mouse spleen cells (5 x 10^5 cells). After 2 h, the cells were stimulated with 30 μg/ml of MBP and incubated for 96 h. Proliferation was assessed by [^{3}H]-thymidine incorporation. Data from one of two representative experiments are presented as CPM ± S.D. of quadruplicate cultures. The CPM for unstimulated media treated cells were 6310 ± 911.


IL-10
TGFβ
TGFβ+IL-10
CPM
100000
10000
1000
100
0.0
0.1
1.0
10.0
100.0
Concentration (ng/ml)

These finding are consistant with our previous observation that orally administered IFNτ protected mice against EAE in the absence of detectable IFNτ in the circulation (Soos *et al.*, 1997).

The induction of suppressor cells is not unique to IFNτ, as IFNβ also induced suppressor cells in spleen cell cultures. Further, the dose response curves for the two IFNs were similar. Also, these suppressor cells produce suppressor factors that inhibit MBP stimulation of EAE spleen cells. Thus, it is quite likely, then, that type I IFNs in general protect against autoimmune diseases such as MS by induction of suppressor cells and suppressor factors.

As indicated above, IFNτ protected mice against EAE when administered orally even though relatively little IFNτ was found in the circulation (Soos *et al.*, 1997). The gut is lined with over half of the cells of the immune system. The suppressor cells induced by oral IFNτ administration must be mobile, since the autoreactive MBP-specific T cells that are inhibited are themselves mobile, and in fact migrate to the central nervous system to cause EAE in the absence of IFNτ treatment. We have shown that IFNτ-treated mice that are immunized with MBP show little or no lymphocyte infiltration of the CNS (Soos *et al.*, 1997).

The CD4 suppressor T cell produced both IL-10 and TGFβ that acted synergistically to inhibit MBP stimulation of spleen cells from EAE mice. IL-10 and TGFβ have previously been shown to inhibit events associated with autoimmune disease (Chaouat *et al.*, 1995; Rott *et al.*, 1994; Stevens *et al.*, 1994; Johns *et al.*, 1991; Schluesener and Lider, 1989). We have shown that IL-10 can be detected in sera of mice which received prolonged i.p. injections or prolonged oral feeding of IFNτ (Soos *et al.*, submitted). Here we have also demonstrated that IFNτ-induced suppressor cells produce IL-10 and TGFβ to synergistically inhibit MBP-specific T cell proliferation. Thus, the findings here serve as the basis for understanding how type I IFNs exert therapeutic effects in autoimmune neuropathies.

CHAPTER 4
CONCLUSION

IFNτ, a recently discovered IFN, is a type I IFN that has pregnancy recognition hormone activity in ruminants. It possesses similar activities observed for the other type I IFNs, IFNα and IFNβ, but in contrast, IFNτ lacks the toxicity associated with high concentrations of these IFNs in tissue culture and in animal studies. Considering the positive therapeutic value of the related IFNβ for treatment of MS, IFNτ has been examined for its ability to prevent the development of EAE, the animal model for MS. IFNτ has been previously shown to prevent the development and superantigen-induced exacerbation of EAE in the absence of toxicity (Soos *et al.*, 1995a). These studies of IFNτ protection against EAE involved initiation of IFN treatment before MBP immunization or before disease development. IFNτ protected against both acute and chronic, relapsing EAE in mice, however, administration of IFN did not block sensitization, since cessation of treatment resulted in development of EAE (Soos *et al.*, 1997). In order to have potential for treatment of MS in humans, the IFNτ must be effective in treatment of active EAE.

In this study we show that both oral administration and ip injection of IFNτ induced remission in SJL/J mice that had ongoing chronic active EAE disease and protected mice against secondary relapses. IFNτ treatment reversed lymphocyte infiltration and microglial activation in the CNS. IFNτ inhibition of antibody production against MBP may be a contributing mechanism by which IFNτ inhibits further relapses of EAE. proliferation of effector B cells and T cells of EAE mice are inhibited by IFNτ in both chronic and acute forms of EAE. Inhibition of MBP-specific T cell clones and reduced B cell responses could contribute to the reversal of disease and histopathological changes shown. Furthermore, IFNτ can prevent EAE by induction of suppressor cells. Injection of these

suppressor cells into mice delayed the onset of EAE. The suppressor cells were found to produce the inhibitory cytokine IL-10 and TGFβ, which acted synergistically to inhibit MBP activation of T cells from EAE mice. This CD4 T suppressor cell is most likely the Th2 type based on the detection of TGFβ and IL-10 in suppressor cell supernatants. Further, since this suppressor cell is induced by IFNτ and probably also by other type I IFNs in the absence of MBP, it is most likely to be antigen-nonspecific in its effect. In fact, preliminary data suggest that suppressor cell supernatant inhibits mitogen stimulation of mouse spleen cells, and superantigen induced effects were similarly suppressed by CD4 T suppressor cells (Figure 15) and their supernatant (Figure 16) via IL-10 and TGFβ. There was no evidence that non-CD4 T cells, including CD8 cells, possessed suppressor cell activity. This observation is in contrast to some other studies on suppressor cells (Nouri *et al.*, 1991; Mukasa *et al.*, 1994; Blank *et al.*, 1995; Castedo *et al.*, 1993). Other studies have also shown that the immune response is suppressed by antigen-specific CD4 Th2 cells (Karpus and Swanborg, 1991; Nabozny *et al.*, 1991; Martinotti *et al.*, 1995; Smith *et al.*, 1991).

Additional potential mechanisms for IFNτ prevention of EAE could include altered cell migration into the CNS, as well as downregulation of MHC I and II molecules and certain co-stimulatory/adhesion molecules. We have previously shown that type I IFNs inhibit the progression of Daudi B cells through the G1 phase of the cell cycle (Subramaniam *et al.*, 1998). Therefore, the inhibition of the cell cycle of cells in G1 may be a mechanism by which IFNτ could inhibit MBP-specific antibody production directly. Thus, other lines of investigation remain to be explored to completely understand the mechanism by which IFNτ can prevent EAE.

Others have seen similar inhibition of clinical disease and reversal of histopathological changes with IFNβ used as the treatment for EAE mice (Yu *et al.*, 1996). Furthermore, treatment of MS patients with IFNβ *in vivo* as well as treatment of T cells

Figure 15. IFNτ induction of CD4 suppressor T cells inhibit SEA stimulation of naive spleen cells. Mouse spleen cell preparations were either pretreated with media or IFNτ for 24 h. (B) CD4 T cells were separated from both media and IFNτ-treated cultures using an affinity immunocolumn. (C) Also, the CD4 T cells were deleted from other media and IFNτ-treated spleen cell prepartions using specific CD4 monoclonal antibody and complement. Naive mouse spleen cells were incubated with superantigen and the media or IFNτ-treated unfractionated cell preparation, the CD4 T cell fraction, or the non-CD4 T cell fraction. Proliferation was measured in CPM 114h later.

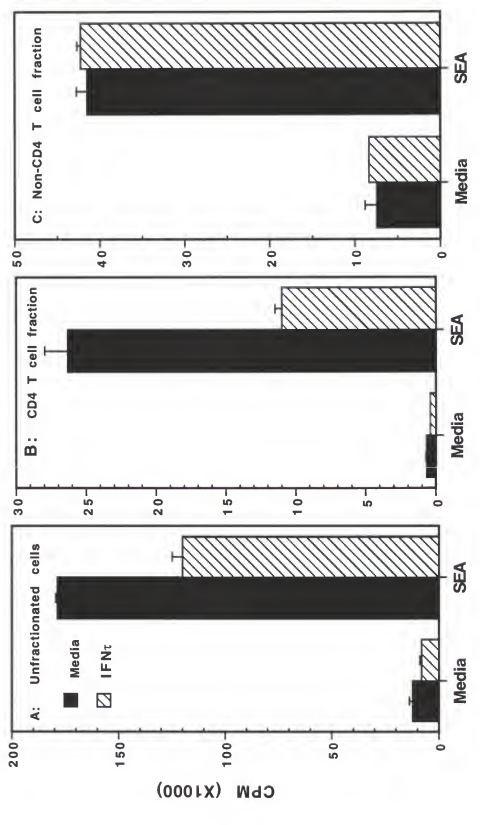
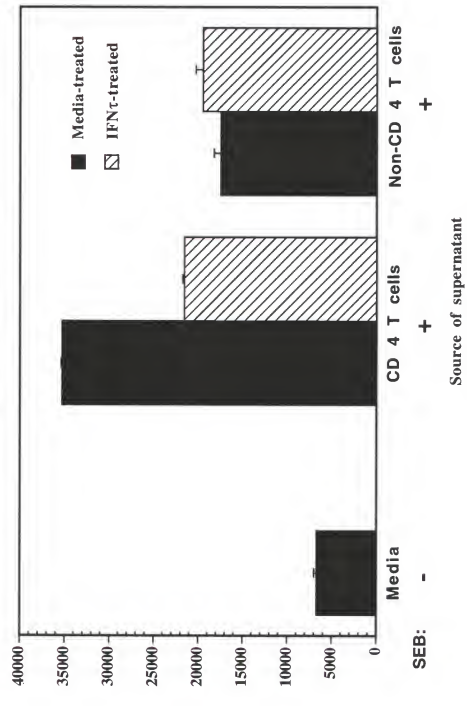

A: Unfractionated cells
B: CD4 T cell fraction
C: Non-CD4 T cell fraction
Media
IFNγ
CPM (X1000)
Media
SEA

Figure 16. IFNτ treated CD4 T cells produce supreesor factor(s) which inhibit SEB stimulation of naive spleen cells. Mouse spleen cell preparations were either pretreated with media or IFNτ for 24 h. CD4 T cells were separated from both media and IFNτ-treated cultures using an affinity immunocolumn. Also, the CD4 T cells were deleted from other media and IFNτ-treated spleen cell prepartions using specific CD4 monoclonal antibody and complement. Cells were culture in media for 24h and supernatants were collected from various prepations. Naive mouse spleen cells were incubated with superantigen and the media or IFNτ-treated unfractionated cell supernatant preparation, the CD4 T cell supernatant fraction, or the non-CD4 T cell supernatant fraction. Proliferation was measured in CPM 114h later.


Media-treated
IFNτ-treated
CPM
40000
35000
30000
25000
20000
15000
10000
5000
0
Media
CD 4 T cells
Non-CD 4 T cells
SEB:
-
+
+
Source of supernatant

in vitro resulted in an inhibition of T cell activation (Noronha *et al.*, 1993; Rudick *et al.*, 1993). But, it was previously shown that the type I IFNs murine IFNα and murine IFNβ induced toxic side effects manifested as flu-like symptoms, fever, nausea and malaise when used as a therapeutic in humans (Degre, 1974; Fent and Zbinden, 1987). Although we have not focused on the lack of toxicity of IFNτ for the mice in these studies, we have previously shown such lack of toxicity in tissue culture and in mice by monitoring for weight loss and bone marrow suppression (Soos *et al.*, 1995a; Soos *et al.*, 1997).

The study here involved the use of ovine IFNτ. There has not been a successful expression of a human IFNτ with similar properties like those of ovine IFNτ. Therefore, current studies are focused on "humanizing" the ovine IFNτ by construction and expression of an ovineIFNτ/human IFNαD chimeric. The chimeric is made up of residues 1-27 of the ovine IFNτ and residues 28-166 of the human IFNαD, and differs from human IFNαD by 15 residues. The chimeric was constructed based on studies showing that the N-terminus of type I IFNs played a central role as to their toxicity or lack thereof (Pontzer *et al.*, 1994; Subramaniam *et al.*, 1995). These recent studies show that the IFNτ/IFNαD chimeric lacks the toxicity associated with IFNαD with human PBMC and mouse splenocytes (Mujtaba *et al.* 1999). Preliminary data show that the IFNτ/IFNαD chimeric also inhibits MBP stimulation of MBP-sensitized spleen cell (Table 2). The IFNτ/IFNαD chimeric suppressed proliferation more effectively than IFNτ but not as effectively as IFNαD. Viabilities were determined and showed that the IFNαD was the most toxic as compared to IFNτ and the IFNτ/IFNαD chimeric. Thus, the IFNτ/IFNαD chimeric may be a better therapeutic for use in human diseases. Overall, the finding reported here indicates that IFNτ is an effective treatment for ongoing active EAE, and this amelioration of disease is mediated by suppressor cells and their synergistically acting suppressor factors such as IL-10 and TGFβ.

Table 2. IFN inhibition of MBP-sensitized spleen cells

IFN (U/ml)	MBP-induced Proliferation (% Inhibition)	Cell Viability (%)
IFNτ (7000 U/ml)	21 ± 6.6	91
IFNτ (15000 U/ml)	42 ± 2.0	89
IFNτ (3000 U/ml)	57 ± 6.1	85
IFNτ/IFNαD chimeric (7000 U/ml)	57 ± 2.4	86
IFNτ/IFNαD chimeric (15000 U/ml)	58 ± 1.1	81
IFNτ/IFNαD chimeric (30000 U/ml)	73 ± 1.3	81
IFNαD (7000 U/ml)	60 ± 2.4	84
IFNαD (15000 U/ml)	75 ± 2.3	83
IFNαD (30000 U/ml)	76 ± 4.6	76

CPM and cell viability values for media-treated spleen cell cultures were 2710 ± 77 and 93%, respectively.

REFERENCES

Ando, D.G., J. Clayton, D. Kono, J.L. Urban, and E.E. Sercarz. 1989. Encephalitogenic T cells in the B10.PL model of experimental allergic encephalomyelitis (EAE) are of the Th-1 lymphokine subtype. Cell. Immunol. 124:132.

Arnason, B.G., B.D. Jankovic, B.H. Waksman, and L. Wennerstein. 1962. Role of the thymus in immune reactions in rats and suppressive effect of thymectomy at birth on reations of delayed (cellular) hypersensivity and the circulating small lymphocyte. J. Exp. Med. 116:177.

Baker, D. J.K. O'Neil, and J.L. Turk. 1991. Cytokines in the central nervous system of mice during chronic relapsing experimental allergic encephalomyelitis. Cell. Immunol. 134:505.

Baron, J.L., J.A. Madri, N.H. Ruddle, G. Hashim, and C.A. Janeway. 1993. Surface expression of α4 integrin by CD4 T cells is required for their entry into brain parenchyma. J. Exp. Med. 177:57.

Baron, S., S.K. Tyring, W.R. Fleishchmann, D.H. Copenhaver, D.W. Nielse, G.R. Klimpel, J. Stanton, and T.K. Hughes. 1991. The interferons: mechanisms of action and clinical applications. J. Amer. Med. Assoc. 266:1375.

Baxenvanis, C.N., G.J. Reclos, C. Servis, E. Anastopoulos, P. Arsenis, A. Katsiyiannis, and N. Matikas. 1989. Peptides of myelin basic protein stimulate T lymphocytes from patients with multiple sclerosis. J. Neuroimmunol. 22:23.

Bazer, F.W., and H.M. Johnson. 1991. Type I conceptus interferons: Maternal recognition of pregnancy signals and potential therapeutic agents. Am. J. Reprod. Immunol. 26:19.

Bazer, F. W., H. M. Johnson, and J. K. Yamamoto. 1989. Effect of ovine trophoblast protein-1 on replication of feline (FIV) and human (HIV) immunodeficiency viruses (abstr). Biol. Reprod. 40 (Suppl.): 63.

Beck, J., P. Rondot, L. Catinot, E. Falcoff, H. Kirchner, and J. Wietzerbin. 1988. Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: Do cytokines trigger off exacerbations? Acta Neuro. Scan. 78:318.

Benveniste, E.N., J.B. Kwoon, W.J. Chung, J. Sampson, K. Pandya, and L. Tang. 1995. differntial modulation of astrocyte cytokine gene expression by TGFβ. J. Immunol. 154:1521.

Benveniste, E. N. 1997. Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. J. Mol. Med. 75:165.

Bever, C.T. and J.N. Whitaker. 1985. Proteinases in inflammatory demyelinating disease. Springer Semin. Immunopathol. 8:235.

Bignami, A., L.F. Eng, D. Dahl, and C.T. Uyeda. 1972. Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. Brain Res. 43:429.

Billiau, A., H. Heremans, and F. Vandekerckhov. 1988. Enhancement of experimental allergic encephalomyelitis in mice by antibodiies against IFNγ. J. Immunol. 140:1506.

Blank, M., I. Krause, D. Buskila, D. Teitelbaum, J. Kopolovic, A. Afek, I. Goldberg, and Y. Shoenfeld. 1995. Bromocriptine immunomodulation of experimental SLE and primary antiphospholipid syndrome via induction of nonspecific T suppressor cells. Cell. Immunol. 162:114.

Bogdan, C., J. Paik, Y. Vodovotz, and C. Nathan. 1992. Contrasting mechanisms for suppression of macrophage cytokine release by transforing growth factor-beta and interleukin-10. J. Biol. Chem. 267:23301.

Brosnan, C.F. and C.S. Raine. 1996. Mechanism of immune injury in multiple sclerosis. Brain Pathol. 6:243

Brostoff, S.W., and D. w. Mason. 1984. Experimental allergic encephalomyelitis: Successful treatment in vivo with a monoclonal antibody that recognizes T helper cells. H. Immunol. 133:1938.

Castedo, M., L. Pelletier, J. Rossert, R. Pasquier, H. Villarroya, and P. Druet. 1993. Mercury-induced autoreactive anti-class II T cell line protects from experimental autoimmune encephalomyelitis by the bias of $CD8^+$ antiergotypic cells in Lewis rats. J. Exp. Med. 177:881.

Chao, C.C., T.W. Molitor, and S. Hu. 1993. Neuroprotective role of IL-4 against activiated microglia. J. Immunol 151:1473.

Chaouat, G., A. Assal-Meliani, J. Martal, R. Raghupathy, J. Elliot, T. Mosmann, and T.G. Wegmann. 1995. IL10 prevents naturally occurring fetal loss in the CBA X DBA/2 mating combinations, and local defect in IL10 production in this abortion-prone combination is corrected by *in vivo* injection of IFNτ. J. Immunol. 154:4261.

Claudio, L., Y. Kress, J. Factor, and C.F. Brosnan. 1990. Mechanisms of edema formation in experimental autoimmune encephalomyelitis. The contribution of inflammatory cells. Am. H. Pathol. 130:1033.

Cogswell, J.P., N. Zeleznik-Le, and. P-Y. Ting. 1991. Transcriptional regulation of the HLA-DRA gen. Crit. Rev. Immunol. 11:87.

Cross, A.H., B. Cannella, C.F. Brosnan, and C.S. Raine. 1990. Homing to central nervous system vasculature by antigen-specific lumphocytes. Lab. Invest. 63:162.

Cross, J.C., and R. M. Roberts. 1991. Constitutive and trophoblast expression of a class of bovine interferon genes. Proc. Natl. Acad. Sci. USA 88:3817.

D'Amelio, F.E., M.E. Smith, and L.F. Eng. 1990. Sequence of tissue responses in the early stages of experimental allergic encephalomyelitis (EAE): immunohistochemical, light microscopic, and ultrastructural observations in the spinal cord. Glia 3:229.

Degre, M. 1974. Influence of exogenous interferon on the peripheral white blood cell count in mice. Int. J. Cancer 14:699.

Dorr, R.T. 1993. Interferon-a in malignant and viral diseases. Drugs 45:177.

Duong, T.T., J. St. Louis, J.J. Gilber, F.D. Finkerlman, G.H. Strejan. 1992. Effect of anti-interferon γ and anti-interleukin-2 monoclonal antibody treatment on the development of actively and passively induced experimental allergic encephalomyelitis in the SJL/J mouse. J. Neuroimmunol. 36:105.

Ebers, G.C. D.E. Bulman, A.D. Sadovnick, D.W. Paty, S. Warren, W. Hader, T.H. Murray, T.P. Seland, P. Duquette, T. Grey. 1986. A population-based study of multiple sclerosis in twins. N. Engl. J. Med. 315:1638.

Fabry, Z., M.M. Waldschmidt, D. Hendrickson, *et al.* 1992. Adhesion molecules on murine brain microvascular endothelial cells: Expression and regulation of ICAN-1 and Lgp 55. J. Neuroimmunol. 36:1

Farrar, M.A., and R.D. Schreiber. 1993. The molecular cell biology of interferon- γ and its receptor. Ann. Rev. Immunol. 11:571.

Fent, K. and G. Zbinden. 1987. Toxicity of interferon and interleukin. Trends Pharm. Sci. 8:100.

Fierz, W., B. Endler, K. Reske, H. Wekerle, and A. Fontana. 1985. Astrocytes as antigen presenting cells. I. Induction of Ia antigen expression on astrocytes by T cells via immune interferon and its effect on antigen presentation. J. Immunol. 134:3785.

Fiorentino, D.F., M.W. Bond, and T.R. Mosmann. 1989. Two types of mouse helper T cell. IV. Th2 clones secrete a factor that inhibits cytoline production by Th1 clones. J. Exp. Med. 170:2081.

Fleischmann, W.R., S. Koren, and C.M. Fleischmann. 1992. Orally administered interferons exert their white blood cell suppressive effects via a novel mechanism. Proc. Soc. Exp. Bio. Med. 201:200.

Fontana, A., W. Fierz, and H. Wekerle. 1984. Astrocytes present myelin basic protein to encephalitogenic T- cell lines. Nature 307:273.

Gasser, D.L., C.M. Newlin, J. Palm, and N.K. Gonatas. 1973. Benetic control of susceptibility to experimental allergic encephalomyelitis in rats. Science 181:872.

Gastle, G., and C. Huber. 1988 The biology of interferon actions. Blut 56:193-199

Gay, D. and M. Esiri. 1991. Blood-brain barrier damage in acute multiple sclerosis plaques: An immunocytological study. Brain 114:557.

Genain, C.P., B. Cannella, S.L. Hauser, and C.S. Raine. 1999. Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nature med. 4(2):170.

Giulian, D., and L.B. Lachman. 1985. Interleukin-1 stimulation of astroglial proliferation after brain injury. Science 228:497.

Gerritse, K., C. Deen, M. Fasbender, R. Ravid, W. Boersma, and E. Claassen. 1994. The involvement of specific anti myelin basic protein antibody-forming cells in multiple sclerosis immunopathology. J. Neuroimmunol. 49:153.

Goverman, H., A. Woods, L. Larson, L.P. Weiner, L. Hood, and D.M. Zaller. 1993. Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. Cell 72:551.

Gresser, I., M. Bandu, D. Brouty-Boye, and M.G. Tovey. 1974. Pronounced antiviral activity of human interferon on bovine and porcine cells. Nature 251:534.

Grossberg, S.E. 1972. the interferons and their inducers: Molecular and therapeutic considerations. N. Engl. J. Med. 287:13.

Gutterman, J.U. 1994. Cytokine therapeutics: Lessons from interferon α. Proc. Natl. Acad. Sci. USA 91:1198.

Hafler, D.A. and H.L. Weiner. 1989. MS: A CNS and systemic autoimmune disease. Immunol. Today 10(3):104.

Hashim, G.A. and M. Brewen. 1985. Myelin basic protein-responsive blood T lymphocytes in patients with multiple sclerosis. J. Neurosci. Res. 13:349.

Hauser, S.L., A.K. Bhan, and F.H. Gilles. 1983. Immunohistochemical staining of human brain with monoclonal antibodies that identify lymphocytes, monocytes and the Ia antigen. J. Neuroimmuol. 5:197.

Heeke, G. V., T.L. Ott, A. Strauss, D.Ammaturo, and F.W. Bazer. 1996. High yield expression and secretion of the ovine pregnancy recognition hormone interferon-tau by *Pichia pastoris.* J. Interferon Res. 16:119.

Hickey, W. F. and H. Kimura. 1988. Perivascular microglial cells of the CNS are bone moarrow-derived and present antigen in vivo. Science 239:290.

Hirsch, R.L., H.S. Panitch, and K.P. Johnson. Lumphoctyes from multiple sclerosis patients produce elavated level of gamma interferon in vitro. J. Clin. Immunol. 5:386.

Hoffman, F.M., D.R. Hinton, J. Baemayr, M. Weil, and H.E. Merrill. 1991. Lymphokines and immunoregulatory molecules in subacute sclerosing panencephalitis. Clin. Immunol. Immunopathol. 58:331.

Hoffman, F.M., R. VonHanwher, C. Dinarello, S. Mizel, D. Hinton, and J.E. Merrill. 1986. Immunoregulatory molecules and IL-2 receptors identified in multiple sclerosis brain. J. Immunol. 136:3239.

Houghton, A.N., T.M. Thomson, D. Gross, H.F., Oettgen, and L.J.Old. 1984. Surface antigens of melanoma and melanocytes: Specificity of induction of Ia antigens by human γ-interferon. H. Exp. Med. 160:255.

Howell, M.D., S.t. Winters, T. Olec, D. Carlo, and S.W. Brostoff. 1989. Vaccination against EAE with a synthetif T cell receptor idiotype. In Workshop on Genes and Supseptibility to MS(Abstr.) Cambridge, England, July 29, 1989.

IFNβ Multiple Sclerosis Study Group. 1993. Interferon β-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo controlled trial. Neurology 43(4):655.

Imakawas, K., R.V. Anthony, M. Kazemi, K.R. Marotti, H.G. Polites, and R.M. Roberts. 1987. Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophectoderm. Nature 330:377.

Isaac, A., and J. Lindenmann. 1957. Virus interference I: The interferon. Proc. R. Soc. Ser. B. 147:258.

Jacobs, C.A., P.E. Baker, E.R. Roux, *et al.* 1991. Experimental autoimmune encephalomyelitis is exacerbated by IL-1a and suppressed by soluble IL-1 receptor. J. Immunol. 146:2983.

Johns, L.T., K.C. Flanders, G.E. Ranges, and S. Sriram. 1991 Successful treatment of experimental allergic encephalomyelitis with transforming growth factor-β1. J. Immunol. 147:1792.

Johnson, H.M., F.W. Bazer, B.S., Szente, and M.A. Jarpe. 1994. How interferons fight disease. Sci. Am. 270:68.

Johnson, H.M., and J.E. Blalock. 1980 Interferon immunosuppression: mediation by a suppressor factor. Infect. Immun. 29:301.

Karpus, W.J., and R.H. Swanborg. 1991. $CD4^+$ suppressor cells inhibit the function of effector cells of experimental autoimmune encephalomyelitis through a mechanism involving transforming growth factor-beta. J. Immunol. 146:1163.

Kennedy, M.K., D.S. Torrance, K.S. Picha, and K.M. Hohler. 1992 Analysis of cytokine mRNA expression in the central nervous system of mice with experimental autoimmune encephalomyelitis reals that IL-10 mRNA expression correlates with recovery. J. Immunol. 149:2496.

Khoury, S.J., W.W. Hancock, and H.L. Weiner. 1992. Oral tolerance to myelin basic protein and antural recovery from experimental autoimmune encephalomyelitis are associated with downregulation of inflammatory cytokines and differential upregulation of transforming growth fact β, interleukin 4, and prostaglandin E expression in the brain. J. Exp. Med. 176:1355.

Kuchroo, V.K., C.A. Martin, H.M. Greer, S-T Hy, R.A. Sobel, and M.E. Dorf. 1993. Cytokines and adhesion moecules contribute to the ability of myelin proteolipid protein-spefic T cell clones to mediate experimental allergic encephalomyelitis. J. Immunol. 151:4371.

Kumar, V., and E. Sercarz. 1994. Holes in the T cell repertoire to myelin basic protein owing to the absence of the D beta 2-j beta 2 gene cluster: Implications for T cell receptor recognition and autoimmunity. J. Exp. Med. 179:1637.

Kuroda, Y., and Y. Shimamoto. 1991. Human tumor necrosis factor a augments experimental allergic encephalomyelitis in rat. J. Neuroimmunol 34:159.

Langer, H.A., and S. Pestka. 1988. Interferon receptors. Immunol. Today 9:393.

Lassmann, H. 1998. Neuropathology in multiple sclerosis: New concepts. Mult. Scler. 4:93.

Leibowitz, S., and L. Kennedy. 1972. Cerbral vascular permeability and cellular infiltration in experimental allergic encephalomyelitis. Immunology 22:859.

Lemire, J. M. and W.O. Wigle. 1986. Passive transfer of experimental allergic encephalomeylitis by myelin basic protein-specific L3T4+ cell clones possessing several functions. J. Immunol. 137:3169.

Male, D., G. Pryce, and C. Hughes 1990. Lymphocyte migration into brain modelled in vitro: Control by lymphocyte activation, cytokines and antigen. Cell. Immunol. 127:1.

Mannie, M.D., C.A. Dinarello, and P.Y. Paterson. 1987. Interleukin1 and myelin basic protein synergistically augment adoptive transfer activity of lymphocytes mediating experimental autoimmune encephalomyelitis in Lewis rats. J. Immunol. 138:4229.

Mantovani, A., and E. Dejana. 1989. Cytokines as communication signals between leukocytes and endothelial cells. Immunol. Today 10:370.

Martin, R., H.F. McFarland, and D.E. McFarlin. 1992. Immunological aspect of demyelinating diseases. Annu. Rev. Immunol. 10:153.

Martiney, H.A., M. Litwak, J.W. Berman, J.C. Arezzo, and C.F. Brosnan. 1990. Pathophysiologic effect of interleukin-1β in the rabbit retina. Am. J. Pathol. 137:1411.

Martinotti, A., A. Stoppacciaro, M. Vagliani, C. Melani, F. Spreafico, M. Wysocka, G. Parmiani, G. Trinchieri, and M.P. Colombo. 1995. CD4 T cells inhibit *in vivo* the CD8-mediated immune response against murine colon carcinoma cells transduced with interleukin-12 genes. Eur. J. Immunol. 25:137.

McCarron, R.M., M. Spatz, and O. Kempski. 1986. Interaction between myelin basic protein-sensitized T lymphocytes and murine cerebral vascular endothelial cells. J. Immunol. 137:3428.

McCarron, R.M., L. Wang, M.K. Racke, D.E. McFalin, and M. Spatz. 1993. Cytokine-regulated adhesion between encephalitogenic T lymphocytes and cerebrovascular endothelial cells. J. Neuroimmunol. 43:23.

McFarland, H.F. 1998. The lesion in multiple sclerosis: Clinical, pathological, and magnetic resonance imaging considerations. J. Neuro. Neurosurg. Psychiatry 64:26.

McKinnon, R.D., G. Piras, J. Ida, and M. Dubois-Dalcq. 1993. A role for TGFβ in oligodendrocye differentiation. J. Cell Biol. 121:1397.

Merrill, J.E., D.H. Kong, J. Clayton, D.G. Ando, D.R. Hinton, and F.M. Hoffman. 1992. Inflammatory leukocytes and cytokine in the peptide-induced disease of experimental allergic encephalomyelitis in SJL and B10.PL mice. Proc. Ntl. Acad. Sci. USA 89:574.

Mews, I., M. Bergmann, S. Bunkowski, F. Gullotta, and W. Bruck. 1998. Oligodendrocyte and axon pathology in clinically silent multiple sclerosis lesions. Mult. Scler. 4(2):55.

Morganti-Kossmann, M.C., T. Kossmann, M.E. Brandes, S.E. Mergenhagen, and S.M. Wahl. 1992. Autocrine and paracrine regulation of astrocyte function by transforming growth factor β. H. Neuroimmunol. 39:163.

Mosmann, T.R. and R.L. Coffman. 1989. Th1 and Th2 cells: Different patterns of lymphokine secretion lead to different functional properties. Ann. Rev Immunol. 7:145.

Mujtaba, M. G., J. M. Soos, and H. M. Johnson. 1997. CD4 T suppressor cells mediate interferon tau protection against experimental allergic encephalomyelitis. J. Neuroimmunol. 75:35.

Mujtaba, M.G., L. Villarete, and H.M. Johnson. 1999. IFNτ Inhibits IgE production in a murine model of allergy and in a IgE-producing myeloma cell line. J. Allergy and Clin. Immunol. (In Press).

Mukasa, A., C. Hiramine, and K. Hojo. 1994. Generation and characterization of a continuous line of CD8⁺ suppressively regulatory T lymphocytes which down-regulates experimental autoimmune orchitis (EAO) in mice. Clin. Exp. Immunol. 96:138.

Nabozny, G.H., S.P. Cobbold, H. Waldmann, and Y.L. Kong. 1991. Suppression in murine experimental autoimmune thyroiditis: *In vivo* inhibition of CD4+ T cell-mediated resistance by a nondepleting rat CD4 monoclonal antibody. Cell. Immunol. 138:185.

Noronha, A., A. Toscas, and M. Jensen. 1993. Interferon β decreases T cell activation and interferon γ production in multiple sclerosis. J. Neuroimmunol. 46:145.

Nouri, A.K.T., M. Lombard, and R. Williams. 1991. High serum levels of CD8 antigen in primary biliary cirrhosis: A possible cause of suppressor cell dysfunction. Clin. Exp. Immunol. 86:140.

Paul, N.J., and N.H. Ruddle. 1988. Lymphotoxin. Ann. Rev. Immunol. 6:407.

Pestka, S., H.A. Langer, K.C. Zoon, and C.E. Samuel. 1987. Interferons and their actions. Ann. Rev. Biochem. 56:727.

Peterson, P.Y., and R.H. Swanborg. 1988. Demyelinating diseases of the central and periperal nervous systems. In. Immunological Diseases, 4th ed. M. Samter, D.W. Talmage, M.M. Frank, K.F. Austen, and H.N. Claman, eds. Little Brown, Boston, MA, p. 1877.

Pettinelli, C.B., and D.E. McFarlin. 1981. Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after in vitro activation of lymph node cells by myelin basic protein: Requirement for Lyt 1+2- T lymphocytes. J. Immunol. 127:1420.

Piddlesden, S.J., H. Lassmann, F. Zimprich, B.P. Morgan, and C. Linington. 1993. The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement. Am. J. Pathol. 143:555.

Pontzer, C.H., T.L. Ott, F.W. Bazer, and H.M. Johnson. 1994. Structure/function studies with interferon-tau: evidence of multiple active sites. J. Interferon Res. 14:133.

Pontzer, C.H., F.W. Bazer, and H.M. Johnson. 1991. Antiproliferative activity of a pregnancy recognition hormone, ovine trophoblast protein-1. Cancer. Res. 51:5304.

Pontzer, C.H., B.A. Torres, J.L. Vallet, F.W.Bazer, and H.M. Johnson. 1988. Antiviral activity of the pregnancy recognition hormone ovine trophoblast protein-1. Biochem. Biophys. Res. Commun. 152:801.

Powell, M.B., D. Mitchell, H. Lederman, *et al.* Lymphotoxin and tumor necrosis factor-alpha production by myelin basic protein-specific T cell clones correlates with encephalitogenicity. Intl. Immunol. 2:539.

Prineas, J.W. 1975. Pathology of the early lesion in multiple sclerosis. Hum. Pathol. 6:531.

Prineas, J.W., and R.G. Wright. 1978. Macrophages, lymphoctyes and plasma cells in the perivascular compartment in chronic multiple sclerosis. Lab. Invest. 38:409.

Pulver, M. S. Carrel, J.P. Mach, and N. de Tribolet. 1987. Cultured human fetal astrocytes can be induced by interferon-γ express HLA-DR. J. Neuroimmunol. 14:123.

Raine, C.S., and M.B. Bornstein. 1970. Experimental allergic encephalomyelitis: An ultrastructural study of demyelination in vitr. H. Neuropathol. Exp. Neurol. 29:177.

Raine, C.S., A. Hummelgard, E. Swanson, and M.B. Bornstein. 1973. Multiple sclerosis serum-induced demyelination in vitro. A light and electron microscope study. H. Neurol Sci. 20:127.

Raine, C.S., F. Mokhtarian, and D.E. McFarlin. 1984. Adoptively transferred chronic relapsing experimental autoimmune encephalomyelitis in the mouse: Neurophathologic analysis. Lab. Invest. 51:534.

Raine, C.S., S.C. Lee, L.C. Scheinberg, A.M. Duijvestijn, and A.H. Cross. 1990a. Adhesion molecules on endothelial cells in the central nervous system: An emerging area in the neuroimmunology of multiple sclerosis. Clin. Immunol. Immunopathol. 57:173.

Raine, C.S., B. Canella, A.M. Duijvestin, and A.H. Cross. 1990b. Homing to the central nervous system by antigen-specific lymphocytes. II. Lymphocyte/endothelial cell adhesion during the intial stages of autoimmune demyelination. Lab. Invest. 63:476.

Robbins, D.S., Y. Shirazi, B.E. Drysdale, A. Lieberman, H.S. Shin, and M.L. Shin. 1987. Production of cytotoxic factor for oligodendrocytes by stimulated astrocytes. J. Immunol. 139:2593.

Roberts, R.M., J.C. Cross, and D.W. Leaman. 1992. Interferons as hormones of pregnancy. Endocr. Rev. 13:432.

Rott, O., B. Fleischer, and E. Cash. 1994. Interleukin-10 prevents experimental allergic encephalomyelitis in rats. Eur. J. Immunol. 24:1434.

Rudick, R.A., C.S. Carpenter, D.L. Cookfair, V.K. Tuohy, and R.M. Ransohoff. 1993. Neurology 43:2080.

Saoudi, A., S. Simmonds, I. Haitinga, and D. Mason. 1995. Prevention of experimental allergic encephalomyelitis in rats by targeting changes in the cytokine response and migratory properties of the autoantigen-specific T cells. J. Exp. Med. 182:335.

Schluesener, H.J., and O. Lider. 1989 Transforming growth factors β1 and β2: Cytokines with identical immunosuppressive effects and potential role in the regulation of autoimmune T cell function. J. Neuroimmunol. 24:249.

Schluesener, H.J. 1990. Transforming growth factors type β1 and β 2 suppress rat astrocyte autoantigen presentation and antagoinze hyperinduction of class II major histocompatability complex antigen expression by interferon-γ and tumor necrosis factor-α. J. Neuroimmunol. 27:41.

Schwartz, R., F. Momburg, G. Moldenhauer, B. Dorken, and V. Schirrmacher. 1985. Induction of HLA class-II antigen expression on human carcinoma cell lines by IFN-gamma. Int. J. Cancer 35:245.

Selmaj, K.W., M. Farooq, W.T. Norton, C.S. Raine, and C.F. Brosnan. 1990. Proliferation of astrocytes in vitro in response to cytokines. A primary role for tumor necrosis factor. J. Immunol. 144:129.

Selmaj, K.W., and C.S. Raine. 1988. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann. Neurol. 23:339.

Selmaj, K., C.S. Raine, and A.H. Cross. 1991. Anti-tumor necrosis factor therapy abrogates autoimmune demyelination. Ann. Neurol. 30:694.

Selmaj, K., C.S. Raine, M. Farooq, W.T. Norton, and C.F. Brosnan. 1991. Cytokine cytotoxicity against oligodendrocytes: Apoptosis induced by lymphotoxin. J. Immunol. 147:1522.

Sen, G.C., and P. Lengyel. 1992. The interferon system. J. Biol. Chem. 267(8):5017.

Smith, H., Y. Sakamoto, K. Kasai, and K.S. Tung. 1991. Effector and regulatory cells in autoimmune oophoritis elicited by neonatal thymectomy. J. Immunol. 147:2928.

Soos, J. M., and H. M. Johnson. 1995b. Type I interferon inhibition of superantigen-induced stimulation: Implications for the treatment of superantigen-associated disease. J. IFN Cytokine Res. 15:39.

Soos, J.M., M.G. Mujtaba, P.S. Subramaniam, W.J. Streit, and H.M. Johnson. 1997. Oral feeding of interferon τ can prevent the acute and chronic relapsing forms of experimental allergic encephalomyelitis. J. Neuroimmunol. 75:43.

Soos, J.M., P.S. Subramaniam, A.C. Hobeika, J. Schiffenbauer, and H.M. Johnson. 1995a. The IFN pregnancy recognition hormone, interferon tau, blocks both development and superantigen reactivation of experimental allergic encephalomyelitis without associated toxicity. J. Immunol. 155:2747.

Steinman, L., D. Solomon, S. Zamvil, K. Lim., and S. Sriram. 1983. Prevention of EAE with anti-I-A antibody: Decreased accumultion of radiolabeled lymphocytes in the central nervous system. J. Neuroimmunol. 5:91.

Stern, A.S., F.J. Podlaski, and J.D. Hulmes. 1990. Purification to homogeneity and partial characterization of cytotoxic lymphocyte maturation factor from human B-lymphoblastoid cells. Proc. Natl. Acad. Sci. USA 87:6808.

Stevens, D.B., K.E. Gould, and R.H. Swanborg. 1994. Transforming growth factor-beta 1 inhibits tumor necrosis factor-alpha/lymphotoxin production and adoptive transfer of disease by effector cells of autoimmune encephalomyelitis. J. Neuroimmunol. 51:77.

Stoll, G., S. Muller, and B. Schmidt. 1993. Localization of interfeon gand Ia-antigen In T cell line-mediated experimental autoimmune encephalomyelitis. Am J. Pathol. 142:1866.

Streit, W. J., and C. A. Kincaid-Colton. 1995. The brain's immune system. Sci. Am. 273:38.

Streit, W. J., M. B. Graeber, and G. W. Kreutzberg. 1988. Functional Plasticity of microglia: A review. GLIA 1:301.

Stuart, G., and K.S. Krikorian. 1928. The neuro-paralytic accidents of anti-rabies treatment. Ann. Trop. Med. Parasitol. 22:327.

Subramaniam, P.S., P.E. Cruz,. A.C. Hobeika, and H.M. Johnson. 1998. Type I interferon induction of the Cdk-inhibitor p21WAF1 is accompanied by ordered G1 arrest, differentiation and apoptosis of the Daudi B-cell line. Oncogene 16:1885.

Subramaniam, P.S., S.A. Khan, C.H. Pontzer, and H.M. Johnson. Differential recognition of the type I interferon receptor by interferon tau and alpha is reponsible for their disparate cytotoxicities. Proc. Natl. Acad. Sci. USA 92:12270.

Sun, J. B. 1993. Autoreactive T and B cells in nervous system diseases. Acta Neuro. Scand. Suppl. 142:1

Swanborg, R.H. 1995 Experimental autoimmune encephalomeylitis in rodents as a model for human demyelinating disease. Clin. Immuno. Immunopathol. 77:4.

Suzumura, A., S.G.E. Mezitis, N.K. Gonatas, and D.H. Silberberg. 1987. MHC antigen expression on bulk isolated macrophage-microglia from newborn mouse brain: Induction of Ia antigen expression by γ-interferon. J. Neuroimmunol. 15:263.

Suzumura, A., M. Sawada, H. Yamamoto, and T. Marunoouchi. 1993. Transforming growth factor-β suppresses activation and proliferation of microglia in vitro. J. Immunol. 151:2150.

Symons, J.A., R.V. Bundick, A.J. Suckling, and M.G. Rumsby. 1987. Cerebrospinal fluid interleukin 1-like activity during chronic relapsing experimental allergic encephalomyelitis. Clin. Exp. Immunol. 68:648.

Tabira, T. 1988. Autoimmune demyelination in the central nervous system. Ann. NY Acad. Sci. 540:187.

Thompson, A., J. Miller, and B. Youl. 1992. Serial gadolinium enhanced MRI in relapsing/remitting multiple sclerosis of varying disease duration. Neurology 42:60.

Traugott, U., and P. Lebon. 1988. Multiple sclerosis: involvement of interferons in lesion pathogenesis. Ann. Neurol. 24:234.

Traugott, U., D.E. McFarlin, and C.S. Raine. 1986. Immunopathology of the lesion in chronic relapsing autoimmune encephalomyelitis in the mouse. Cell. Immunol. 99:395.

Waksman, B.M., S. Arbuys, and B. Arnason. 1961. The use of specific lymphocyte antisera to inhibit hypersensitvie reactions of the delayed type. J. Exp. Med. 114:997.

Wang, Z. Y., B. He, J. Qiao, and H. Link. 1995. Suppression of experimental autoimmune myasthenia gravis and experimental allergic encephalomyelitis by oral administration of acetylcholine receptor and myelin basic protein: double tolerance. J. Neuroimmunol. 63:79.

Warren, K. G., and I. Catz. 1993. Autoantibodies to myelin basic protein within multiple sclerosis central nervous system tissue. J. Neurol. Sci. 115:169.

Wekerle, H. C. Linington, and H. Myeermann, 1986. Cellular immune reactivity within the CNS. Trends Neurosci. 9:271.

Willenborg, D.O. and S.H. Prowse. 1983. Immunoglobulin-deficient rats fail to develop experimental allergic encephalomyelitis. H. Neuroimmunol. 5:99.

Wong, D., and K. Dorovini-Zis. 1992. Upregulation of intercellular adhesion molecule-1 (ICAM-1) expression in primary cultures of human brain microvessel endothelial cells by cytokines and lipopolysaccharide. J. Neuroimmunol 39:11.

Wong, G.H.W., P.F. Bartlett, I. Clark-Lewis, F. Battye, and J.W. Schrader. 1984. Inducible expression of H-2 and Ia antigens on brain cells. Nature 310:688.

Vandenbark, A., G. Hashim, and H. Offner. 1989. Immunization with asynthetic T-cell receptor V-region peptide protects against EAE. Nature 341:541.

Voorthuis, J.A.C., B. Uitdehaag, C. De Groot, P. Goede, P. Meide, and C. Dijkstra. 1990. Suppression of experimetnal allergic encephalomyelitis by intraventricular administration of interferon gamma in Lewis rats. Clin. Exp. Immunol. 81:183.

Yao, J. L. Harvath, D.L. Gilber, and C.A. Colton. 1990. Chemotaxis by a CNS macrophage, the microglia. H. Neurosci. Res. 27:36.

Yong, B.W., R. Moumdijian, F.P. Yong, T.C.G. Ruijs, M.S. Freedman, N. Cashman, and H.P. Antel. 1991. γ-Interferon promotes proliferation of adult human astrocytes in vitro and reactive gliosis in the adult mouse brain in vivo. Proc. Natl. Acad. Sci. USA 88:7016.

Yu, M., A. Nishiyama, B.D Trapp, and V.K. Tuohy. 1996. Interferon-beta inhibits progression of relapsing-remitting experimental autoimmmune encephalomyelitis. J. Neuroimmunol. 64:91.

Zamvil, S S., A. Al-Sabboagh, P.A. Nelson, D. Kaul, M.S. Charles, D.J. Mitchell, L. Steinman, H.L. Weiner, and V.K. Kuchroo. 1994. "Lupus-prone" mice are susceptible to organ-specific autoimmune disease, experimental allergic encephalomyelitis. Pathobiology 62:113.

Zamvil, S.S., D.J. Mitchell, A.C. Moore, K. Kitamura, L. Steinman, and J.B. Rothbard, 1986. T cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. Nature 324:258.

Zamvil, S.S. and L. Steinman. 1990 The T lymphocyte in experimental allergic encephalomyelitis. Ann. Rev. Immunol. 8:579.

Zoon, K.C., D. Miller, J. Bekisz, *et al.* 1992. Purification and characterization of multiple components of human lymphoblastoid interferon α. J. Biol. Chem. 267:15210.

Zuber, P., M.C. Kuppner, and N. Tribolet. 1988. Transforming grOwth factor b2 down-regulates HLA-DR antigen expression on human malignant glioma cells. Eur.J. Immunol. 18:1623

BIOGRAPHICAL SKETCH

Mustafa Ghulam Mujtaba was born in Kabul, Afghanistan, on September 18, 1973, to Ghulam and Uzra Mujtaba. During the war with the communist Soviet Union, the Mujtaba family left Afghanistan for the neighboring country, Pakistan, in 1983 and then to Cape Coral, Florida in June 1984. Mustafa completed his middle school in Cape Coral. The Mujtaba family then moved to Lake City, Florida in 1988 after his father had a job transfer. He graduated from Columbia High School in 1992. Mustafa attended college at the University of Florida majoring in Microbiology and Cell Science. In 1995, he received his Bachelor of Science degree in Microbiology and was accepted by the same department as a graduate student. He was kindly taken into the laboratory of Dr. Howard Johnson. After completion of his doctoral program, Mustafa plans to pursue research in a similar area as a postdoctoral fellow.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Howard M. Johnson, Chair
Graduate Research Professor of
Microbiology and Cell Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Edward M. Hoffmann
Professor of Microbiology and
Cell Science

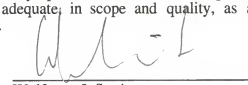
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Julie Maupin
Assistant Professor of
Microbiology and Cell Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Janet K. Yamamoto
Associate Professor of
Veterinary Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Wolfgang J. Streit
Professor of Neuroscience

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1999

Dean, College of Agriculture

Dean, Graduate School